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Comprehensive Evaluation of How TPMT Genotype Influences Thiopurine Treatment

Tanya R Yakushi[1]*, Yong Qu[1], Mike M Moradian[2], Ruan T Ramjit[3]

1 Biochemical Genetics Laboratory, Regional Molecular Genetic Pathology Laboratory, SCPMG Regional Reference Laboratories
2 Director of Operations, Regional Molecular Genetic Pathology Laboratory, SCPMG Regional Reference Laboratories
3 Physician Director, Laboratory Director, Regional Molecular Genetic Pathology Laboratory, Southern California Permanente Medical Group

Corresponding author: Tanya R Yakushi

tanya.r.yakushi@kp.org

Tel: 818- 502-5924

Biochemical Genetics Laboratory, Regional Molecular Genetic Pathology Laboratory, SCPMG Regional Reference Laboratories, 4580 Electronics Pl, Los Angeles, CA 90039

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Abstract

Purine analogs, 6-mercaptopurine (6-MP) and the prodrug azathioprine (Aza) are used as immunosuppressants in the treatment of many diseases including cancer, autoimmune disorders and inflammatory diseases of the digestive tract. Treatment with thiopurines is complicated by the high variability in response observed in a patient population. The need to titrate treatment to adequate therapeutic levels is exacerbated by the cytotoxicity that can result from overdosing patients. In this comprehensive study, we evaluated the response of 946 individuals, with known thiopurine S-methyltransferase (TPMT) genotypes, to treatment with 6-MP and Aza. We determined the allelic frequencies of the most common TPMT alleles in a diverse cohort of individuals. The TPMT*1/TPMT*1 genotype was found to occur in 92.1% of the patient population, while the TPMT*1/TPMT*3A, TPMT*1/TPMT*3C, and TPMT*1/TPMT*2 genotypes were found to occur in 6.0%, 1.8%, and 0.1% of the patient population, respectively. We evaluated how genotype affected therapeutic response and make safe dosing recommendation based on genotype. The observations made in this study, strongly suggests a need to prescribe patients with the TPMT*1/TPMT*3A genotype ~50% of the dose prescribed to wild type individuals and ~25% of the TPMT*1/TPMT*1 dosage to individuals encoding the TPMT*1/TPMT*3C genotype. The results presented are intended to serve as a guide to better understand the complex relationship between genotype and pharmaceutical response to thiopurine drugs.

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Introduction

Thiopurine S-methyltransferase (TPMT) is a critical enzyme in the metabolism of thiopurine drugs, 6- mercaptopurine (6-MP) and azathioprine (Aza) [1-2]. Thiopurines are used to treat many diseases including acute lymphoblastic leukemia, autoimmune disorders such as rheumatoid arthritis and autoimmune hepatitis, and inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, as well as immunosuppressants after organ transplantation [3-6]. Thiopurines exert their cytotoxic effects through a multistep conversion into 6- thioguanine nucleotides (6-TGNs), the presence of which disrupt DNA replication and rapidly growing cells, [2,7- 8]. TPMT is a methyltransferase that covalently attaches a methyl group to thiopurine metabolites thereby shunting them from their eventual conversion into active cytotoxic 6-TGNs [1]. [Figure 1] summarizes the conversion of thiopurines into active 6-TGNs.

Individual TPMT activity varies due to the occurrence of specific single nucleotide polymorphisms that result in low or deficient TPMT activity. Previous studies have shown that polymorphisms give rise to a population in which approximately 1 in 300 individuals are deficient in TPMT activity, 10% of individuals exhibit intermediate activity, while the rest of the population displays wild type levels [9-13]. Individuals homozygous for the wild type allele (*1) exhibit normal to high TPMT activity. Heterozygous individuals carrying one functional allele and one nonfunctional allele (common nonfunctional alleles include *2, *3A, *3B, or *3C) exhibit intermediate activity, while individuals homozygous for nonfunctional alleles exhibit low to deficient activity [14-18]. Reduced TPMT activity can result in the over accumulation of cytotoxic thiopurine metabolites which lead to adverse secondary effects, such as myelosuppression, leukopenia, pancreatitis, and gastrointestinal intolerance, the effects of which are exacerbated in individuals with low or deficient activity [18-20]. Spire-Vayron

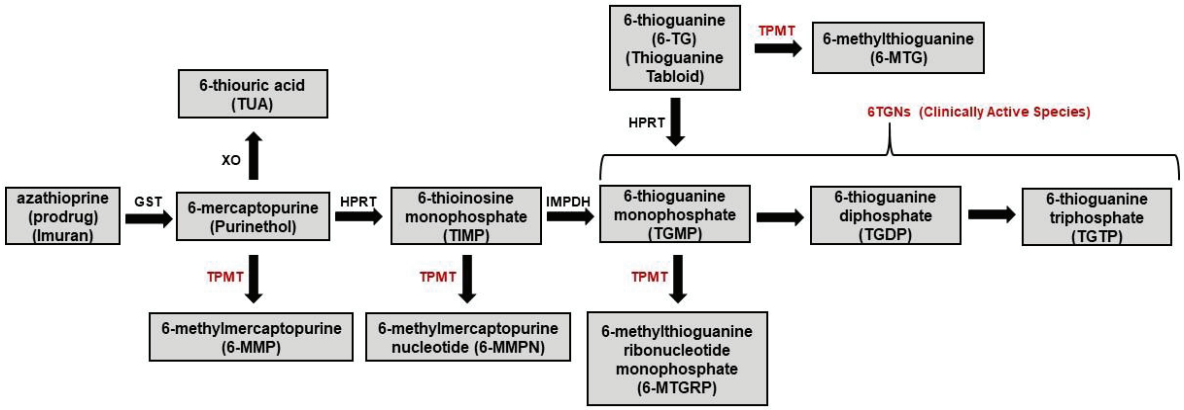


Figure 1 Thiopurine metabolism overview. The figure provides an overview of the main metabolites in the metabolism of the pro-drug azathioprine and 6-mercaptopurine.

de la Moureyre et al. [10] demonstrated that even within a genotype category, the phenotypic response to treatment can vary widely. Interestingly, the variability in response is greatly reduced in heterozygous individuals, indicating that these individuals may have more predictable outcomes to treatment [20-10].

Due to the narrow therapeutic index, individual variation in response to treatment and potential adverse side effects resulting from toxic metabolite accumulation, it is important to monitor metabolite levels during thiopurine treatment [20-21, 8]. Monitoring thiopurine metabolite accumulation assists in evaluating adherence to therapy, preventing cytoxicity and allows for the continued titration of therapy. Clinically, thiopurine treatment is monitored by quantifying thiopurine metabolite levels in red blood cells by liquid chromatography tandem mass spectrometry [13-22]. The complexity of the various metabolites in the pathway is reduced by an acid hydrolysis step that converts many of the metabolites into a few components. Two specific hydrolysis products are measured, 6-thioguanine (6-TG) and 6-methylmercaptopurine (6-MMP). Quantifying 6-TG levels provides a measurement of thiopurine incorporation into 6-TGNs, the active metabolites in thiopurine treatment. Dosing recommendations based on 6-TG and 6-MMP levels have been well established [23-25, 8]. Low levels of 6-TG can indicate nonadherence or the need to increase dosage. High levels of 6-TG may indicate possible toxicity and increased susceptibility to myelosuppression, and the need to decrease or change treatment. 6-MMP is measured to evaluate TPMT function and treatment adherence. Certain individuals have higher than normal TPMT activity and are considered “shunters” or hypermethylators, which results in the excessive conversion of 6-MP and Aza, directly into 6-MMP, the TPMT catalyzed methylated product of 6-MP. As a result, there is a less than expected conversion of 6-MP or Aza into active 6-TGN metabolites. High levels of 6-MMP and low levels of 6-TG indicates that the individual is a shunter and may need treatment supplemented with allopurinol or may benefit from an alternative treatment, while high levels of 6-MMP and high levels of 6-TG indicates overdosing [26-28]. Monitoring to prevent the over accumulation of 6-MMP is important in circumventing hepatotoxicity. Guidelines have been established for therapeutic levels of 6-TG and 6-MMP. The established values are as follows: 6-MMP (<5700 pmol/8x108 RBCs) and 6-TG (235-400 pmol/8x108 RBCs) [29]. [Table 1] summarizes established thiopurine metabolite-directed dosing recommendations [25].

In this study, we have monitored 946 individuals from a diverse patient population, undergoing treatment with purine analogs, 6-MP and Aza for a wide variety of diseases. We have used genotyping and metabolite monitoring data acquired over a year, to evaluate the effect of genotype on treatment. The results obtained allowed us to make genotype specific safe dosing recommendations for patients starting treatment with thiopurine drugs.

Methods

The thiopurine metabolite quantitative assay was performed as described by Dervieux et al., [13] with modifications. The thiopurine metabolite assay is used to monitor metabolite levels in patients undergoing treatment with Imuran (azathioprine, Aza) and Purinethol (6-mercaptopurine, 6-MP) extracted from EDTA treated whole blood. Briefly, packed red blood cells were washed and hydrolyzed in the presence of the reducing agent, dithiothreitol (DTT) under boiling conditions. Hydrolyzed metabolites were fractionation by liquid chromatography on a Shimadzu liquid chromatography system and quantified using a Sciex API4000 triple quadrupole mass spectrometer by selective reaction monitoring. Quantified 6-TG and 6-MMP levels were normalized to red blood cell count and reported in units of pmol/8x108 RBCs.

The laboratory has monitored results for 127 batches, providing 1731 specimen results, for 946 patients with known genotypes. Patient evaluation of thiopurine metabolite values ranged from 1-11 measurements. All results obtained were categorized based on the five categories described in [Table 1]. Results in which the 6-TG values were <235 pmol/8x108 RBCs and 6-MMP values were <5700 pmol/8x108 RBCs were defined as “Underdosed”, results in which 6-TG values were <235 pmol/8x108 RBCs and 6-MMP values were ≥5700 pmol/8x108 RBCs were defined as

Table 1. Dosing categories based on 6-TG and 6-MMP metabolite concentrations. The therapeutic levels adopted by the clinical community are values of 235-400 pmol/8x10^8 RBCs for 6-TG and <5700 pmol/8x10^8 RBCs for 6-MMP. Guidelines were adapted from Vande Casteele et al. (2017).

6-TG Levels (235-400 pmol/ 8x10^8 RBCs)	6-MMP Levels (<5700 pmol/ 8x10^8 RBCs)	Interpretation	Proposed Management
Low (<235)	Normal (<5700)	**Underdosed** or Noncompliant	Increase dose OR if noncompliant, then educate about compliance
Low (<235)	High (≥5700) with 6-MMP/6-TG ratio >11	6-MMP **Shunter**	Change therapy OR add allopurinol and reduce thiopurine dose
Therapeutic (235-400)	Normal (<5700)	**Appropriately Dosed**	Dose is in appropriate range
Therapeutic (235-400)	High (≥5700)	**Treatment Refractory**	Change therapy OR adjust dose accordingly
High (>400)	High (≥5700) or Normal	**Overdosed**	Change therapy OR reduce dose

"Shunter", results in which 6-TG values were between 235-400 pmol/8x108 RBCs and 6-MMP values were <5700 pmol/8x108 RBCs were defined as "Appropriately Dosed", results in which 6-TG values were between 235-400 pmol/8x108 RBCs and 6-MMP values were ≥5700 pmol/8x108 RBCs were defined as "Treatment Refractory", and results in which 6-TG values were >400 pmol/8x108 RBCs irrespective of 6-MMP values were defined as "Overdosed". Genotype and dosing information was obtained from electronic medical records.

Results

Thiopurine treatment is challenging due to many factors including noncompliance, concomitant use of other medication, individual response to treatment, incorrect sample collection not during a trough period, among other reasons. In addition, overdosing can lead to severe cytoxicity and secondary effects which could ultimately result in death. Using information gathered on a large and diverse patient population undergoing thiopurine treatment, we compiled data on individuals with known genotypes. We used this data to evaluate the correlation between dose and genotype to suggest genotype specific safe starting dosages with the aim of improving patient outcome and compliance with thiopurine treatment.

Population Analysis

The patient population studied, 946 individuals had known genotypes. Wild type version 1 (TPMT*1) and single nucleotide polymorphisms with the highest prevalence were included in the analysis (TPMT*2, *3A, *3C). [Figure 2A] summarizes the genotype distribution observed. 92.1% of individuals were found to have the TPMT*1/TPMT*1 genotype, while 6.0% and 1.8% of individuals had the TPMT*1/TPMT*3A and TPMT*1/TPMT*3C genotypes, respectively. Only one person who accounted for 0.1% of the total patient population had the TPMT*1/TPMT*2 genotype. Prevalence observed in the mutational analysis is consistent with previously published results [30].

Response to treatment was compared to genotype and clinical results were binned into dosing categories. As expected, individuals with the TPMT*1/TPMT*1 genotype displayed varying degrees of response to treatment. 1590 results for 871 wild type individuals were categorized into the following categories: "Underdosed" (6-TG <235 pmol/8x108 RBC; 6-MMP <5,700 pmol/8x108 RBCs), "Shunter" (6-TG <235 pmol/8x108 RBC; 6-MMP ≥5,700 pmol/8x108 RBCs), "Appropriately Dosed" (6-TG 235-400 pmol/8x108 RBC; 6-MMP <5,700 pmol/8x108 RBCs), "Treatment Refractory" (6-TG 235-400 pmol/8x108 RBC; 6-MMP ≥5,700 pmol/8x108 RBCs), and "Overdosed" (6-TG >400 pmol/8x108 RBCs), summarized in [Figure 2B]. 60.1% of wild type results were found to be "Underdosed", 6.5% were found to be "Shunters", 20.6% were found to be "Appropriately Dosed", 4.2% of results were categorized as "Treatment Refractory", while 8.5% of results belonged to the "Overdosed" category. Of the results in the "Underdosed" category, 9.3% of results were found to have analyte levels below the Lower Limit of Quantitation (LLOQ), for both 6-TG (<30 pmol/8x108 RBCs) and 6-MMP (<300 pmol/8x108 RBCs). These results belonged to individuals who were either non-compliant, highly underdosed, tested before commencing treatment, or tested after stopping treatment. The 6.5% and 4.2% of results in the "Shunter" and "Treatment Refractory" categories, respectively, belonged to individuals expressing high levels of TPMT activity which resulted in a high conversion of 6-MP or Aza to methylated 6-MP containing byproducts. Results in which 6-TG levels were >400 pmol/8x108 RBCs, irrespective of 6-MMP levels, were classified as "Overdosed" and constituted 8.5% of the total wild type results. These individuals responded aggressively to treatment and required either lowering their dosage or changing treatment to avoid myelotoxicity.

Heterozygous individuals possessing the genotype TPMT*1/TPMT*3A and TPMT*1/TPMT*3C produced a distribution of results very different to what was observed for wild type individuals. Since these patients have reduced TPMT activity, only one result was observed with a 6-MMP value ≥5700 pmol/8x108 RBCs, in a TPMT*1/TPMT*3A individual who was overdosed. Consequently, no results were observed for TPMT*1/TPMT*3A and TPMT*1/TPMT*3C encoding individuals in the "Shunter" or "Treatment Refractory" categories. 99 results were obtained for individuals with the TPMT*1/TPMT*3A genotype, the second largest category whose distribution was studied. Of the results, 27.3% were categorized as "Underdosed", 35.4% were categorized as "Appropriately Dosed" and 37.4% were categorized as "Overdosed" [Figure 2C]. 40 results were obtained for individuals with the TPMT*1/TPMT*3C genotype. Of the results, 45.0% were categorized as "Underdosed", 35.0% were categorized as "Appropriately Dosed" and 20.0% were categorized as "Overdosed" [Figure 2D]. The distribution of results for heterozygous individuals was shifted to a higher percentage of "Appropriately Dosed" and "Overdosed" results,

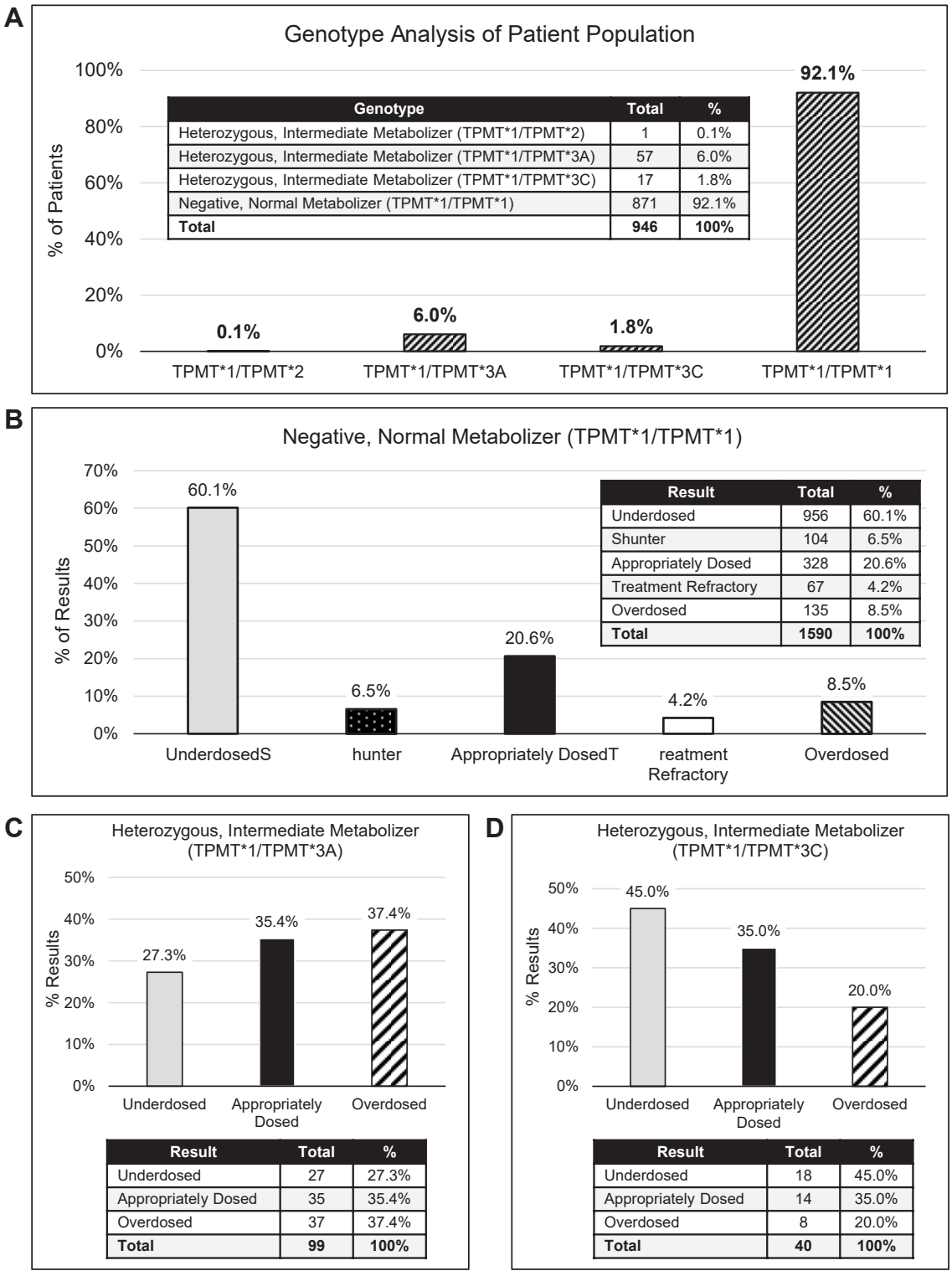


Figure 2 Distribution of patient population with known genotypes. (A) Absolute values and Percentages of individuals identified with TPMT*1/TPMT*2 (1, 0.1%), TPMT*1/TPMT*3A (57, 6.0%), TPMT*1/TPMT*3C (17, 1.8%), and TPMT*1/TPMT*1 (871, 92.1%) genotypes are summarized in the embedded table, while percentages of each genotype are depicted in the bar chart. (B) 1590 results for 871 TPMT*1/TPMT*1 individuals were classified based on quantified 6-TG and 6-MMP analyte values. Results were categorized as Underdosed" (6-TG <235 pmol/8x108 RBC; 6-MMP <5,700 pmol/8x108 RBC), "Shunter" (6-TG <235 pmol/8x108 RBC; 6- MMP ≥5,700 pmol/8x108 RBC), "Appropriately Dosed" (6-TG 235-400 pmol/8x108 RBC; 6-MMP <5,700 pmol/8x108 RBC), "Treatment Refractory" (6-TG 235-400 pmol/8x108 RBC; 6-MMP ≥5,700 pmol/8x108 RBC), and "Overdosed" (6-TG >400 pmol/8x108 RBC). The percentage of results identified in each category are illustrated in the bar chart. (C) The distribution of 99 results for 57 TPMT*1/TPMT*3A individuals and (D) 40 results for 17 TPMT*1/TPMT*3C individuals were categorized based on 6-TG and 6-MMP values, the distribution of results are shown in bar charts.

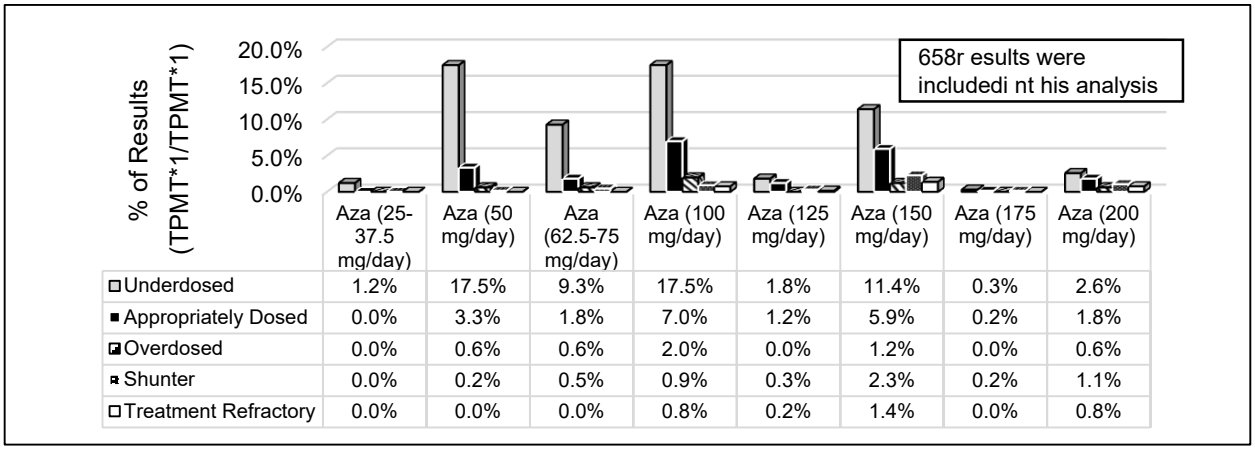
when compared to wild type individuals. The highest category of results for individuals with the TPMT*1/TPMT*3A genotype was found to be in the "Overdosed" category while the highest category of results for individuals with the TPMT*1/TPMT*3C genotype was found to be in the "Underdosed" category.

Genotype Specific Dosing Recommendations

Correlations between optimal thiopurine dosing and genotypes were made, for 6-MP and Aza. Aza is an imidazole derivative of 6-MP, first metabolized in the liver, to produce active 6-MP. Aza and 6-MP are indicated to treat the same diseases, however conversion of Aza to therapeutically active 6-TGN metabolites is slowed due to the additional metabolic step needed to convert Aza to 6-MP [21, 31-32]. Individuals in the proper therapeutic range of 235-400 pmol/8x108 RBCs for 6-TG and <5700 pmol/8x108 RBCs for 6-MMP, with a wild type genotype (TPMT*1/TPMT*1) were evaluated. 1437 results were obtained for 787 individuals with known dosing information. Of the 1437 results, 328 results produced values that were consistent with an "Appropriately Dosed" category, 803 results were consistent with the "Underdosed" category, and 135 results were consistent with the "Overdosed" category. Of the remaining values, 95 results were below the LLOQ for both the 6-TG and 6-MMP analytes, while 171 results produced values consistent with the "Shunter" or "Treatment Refractory" categories. Of note results obtained below the LLOQ for both analytes, were excluded from the dosage distribution analysis. Individuals with this result typically had been tested prior to starting treatment, after stopping treatment or had documented non-compliance; therefore including these results would have inaccurately reflected the effect of an active treatment on response. Categorized results were compared to Aza and 6-MP dosages in the absence of the TPMT mediated inhibitor, allopurinol. The largest percentage of "Appropriately Dosed" results was achieved when Aza was administered at a dose of 100 mg/day [Figure 3A], while the largest percentage of "Appropriately Dosed" results with 6-MP was achieved at a concentration of 50 mg/day [Figure 3B]. These results are consistent with previously published findings that suggest using Aza at approximately 2- fold the amount of 6-MP [33]. A straightforward shift to the "Overdosed" category upon continued increase in dosing was not observed. In some individuals increasing the dose resulted in a sharp increase in 6-MMP levels without a proportional increase in the 6-TG value, causing a shift to the "Shunter" or "Treatment Refractory" categories instead of the "Appropriately Dosed" category.

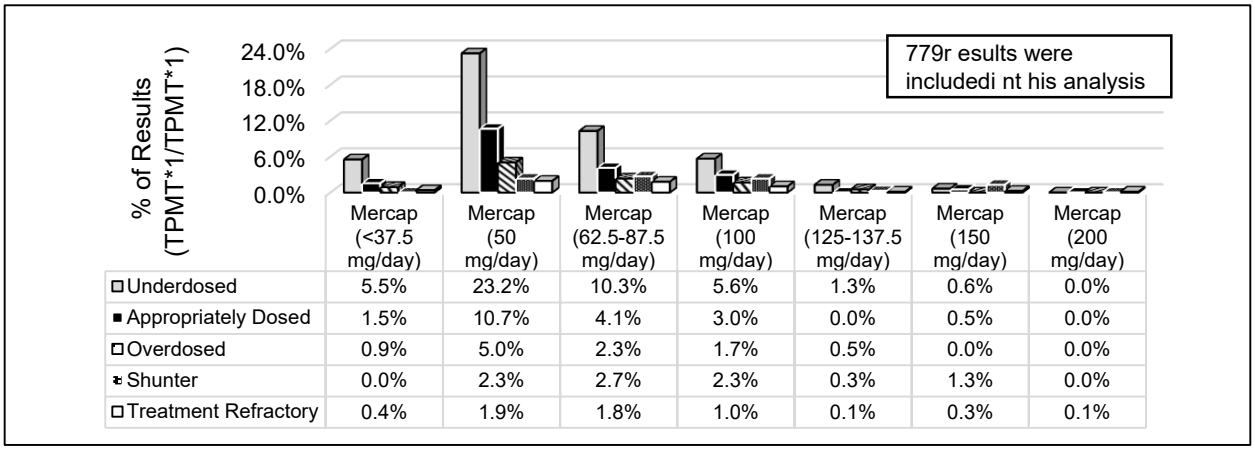
Allopurinol (Allo) is often used in conjunction with 6-MP and Aza when treating individuals who exhibit high TPMT activity [34-36, 8]. Allo mediates the inhibition of TPMT, promoting the conversion of 6-MP and Aza into therapeutically active 6-TGNs, resulting in a decreased accumulation of 6-MMP [37, 28]. A simplified mechanism of action is depicted in Figure 3D. The use of Allo during treatment requires a concomitant decrease in the prescribed dosage of 6-MP and Aza [34, 8]. [Figure 3C and 3E] summarize the distribution of results observed in wild type

A



	Aza (25-37.5 mg/day)	Aza (50 mg/day)	Aza (62.5-75 mg/day)	Aza (100 mg/day)	Aza (125 mg/day)	Aza (150 mg/day)	Aza (175 mg/day)	Aza (200 mg/day)
Underdosed	1.2%	17.5%	9.3%	17.5%	1.8%	11.4%	0.3%	2.6%
Appropriately Dosed	0.0%	3.3%	1.8%	7.0%	1.2%	5.9%	0.2%	1.8%
Overdosed	0.0%	0.6%	0.6%	2.0%	0.0%	1.2%	0.0%	0.6%
Shunter	0.0%	0.2%	0.5%	0.9%	0.3%	2.3%	0.2%	1.1%
Treatment Refractory	0.0%	0.0%	0.0%	0.8%	0.2%	1.4%	0.0%	0.8%

B



	Mercap (<37.5 mg/day)	Mercap (50 mg/day)	Mercap (62.5-87.5 mg/day)	Mercap (100 mg/day)	Mercap (125-137.5 mg/day)	Mercap (150 mg/day)	Mercap (200 mg/day)
Underdosed	5.5%	23.2%	10.3%	5.6%	1.3%	0.6%	0.0%
Appropriately Dosed	1.5%	10.7%	4.1%	3.0%	0.0%	0.5%	0.0%
Overdosed	0.9%	5.0%	2.3%	1.7%	0.5%	0.0%	0.0%
Shunter	0.0%	2.3%	2.7%	2.3%	0.3%	1.3%	0.0%
Treatment Refractory	0.4%	1.9%	1.8%	1.0%	0.1%	0.3%	0.1%

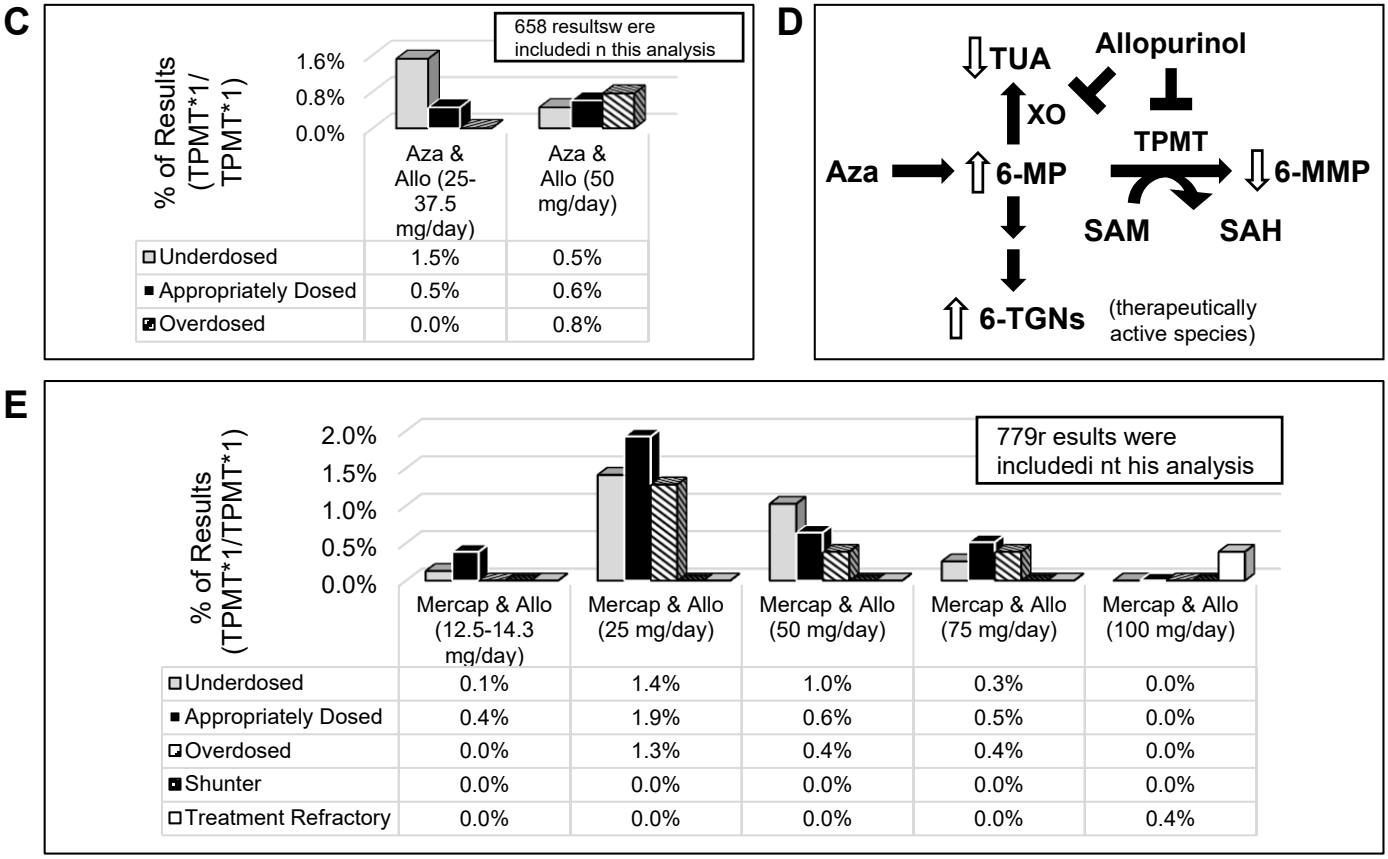


Figure 3 Genotype specific dosing distribution for wild type TPMT*1/TMPT*1 treated individuals. (A) Distribution by percentage of wild type individuals undergoing azathioprine treatment ranging between 25-200 mg/day. Doses higher than 200 mg/day or lower than 25 mg/day of azathioprine were not observed during this analysis. (B) Distribution by percentage of wild type individuals undergoing mercaptopurine treatment ranging between 12.5-200 mg/day. Doses higher than 200 mg/day or lower than 12.5 mg/day of mercaptopurine were not observed during this analysis. (C) Distribution by percentage of wild type individuals undergoing treatment with azathioprine in combination with 100 mg/day of allopurinol. Treatment with azathioprine ranged between 25-50 mg/day. Azathioprine doses outside of that range were not observed. (D) Schematic depicts a simplified overview of allopurinol mediated inhibition of thiopurine S-methyltransferase (TPMT) and direct inhibition of xanthine oxidase (XO), along with the effects observed on metabolite levels, **including thiouric acid (TUA)**. (E) Distribution by percentage of wild type individuals undergoing treatment with mercaptopurine in combination with 100 mg/day of allopurinol. Treatment with mercaptopurine ranged between 12.5-100 mg/day. Mercaptopurine doses outside of that range were not observed.

individuals. Using Allo required decreasing the dose of 6-MP and Aza by 25-50%, consistent with previously published findings [34-36].

As expected, individuals with a heterozygous genotype did not have results categorized in either the shunter or refractory categories, due to their intermediate thiopurine methyltransferase activity. A total of 90 results were obtained for 50 individuals encoding the TPMT*1/TPMT*3A genotype with known dosing information. The largest percentage of "Appropriately Dosed" TPMT*1/TPMT*3A individuals (20.0%) was achieved when a dose of 50 mg/day of Aza was administered [Figure 4A]. Results obtained when 6-MP was administered were mixed [Figure 4B]. 8.0% of TPMT*1/TPMT*3A individuals treated with 6-MP were "Appropriately Dosed" when 25 mg/day was prescribed and 10.0% were "Appropriately Dosed" when 50 mg/day was prescribed. It is important to note that although the largest percentage of "Appropriately Dosed" was observed when 50 mg/day was prescribed, the ratio of "Overdosed" to "Appropriately Dosed" was 1.8:1, indicating that at this dosage the patient is almost twice as likely to be overdosed at this concentration, than "Appropriately Dosed". It stands to reason that the safer starting dose for individuals with a TPMT*1/TPMT*3A genotype is 25 mg/day when 6-MP is selected.

Results for 14 TPMT*1/TPMT*3C individuals were evaluated in 35 analyses. When Aza was prescribed, an equal percentage (10.0%) of TPMT*1/TPMT*3C heterozygous individuals achieved "Appropriately Dosed" levels at 25-28.6 mg/day and 50 mg/day [Figure 4C]. However, at a dosage of 50 mg/day, TPMT*1/TPMT*3C individuals were 3x as likely to be "Overdosed", when compared to 25 mg/day, indicating that the safer starting dosage for TPMT*1/TPMT*3C individuals prescribed Aza is 25 mg/day. Prescribing 6-MP to TPMT*1/TPMT*3C individuals resulted in the largest percentage of "Appropriately Dosed" results being achieved at dosages below 10 mg/day [Figure 4D]. Since fewer measurements were made for TPMT*1/TPMT*3C individuals,

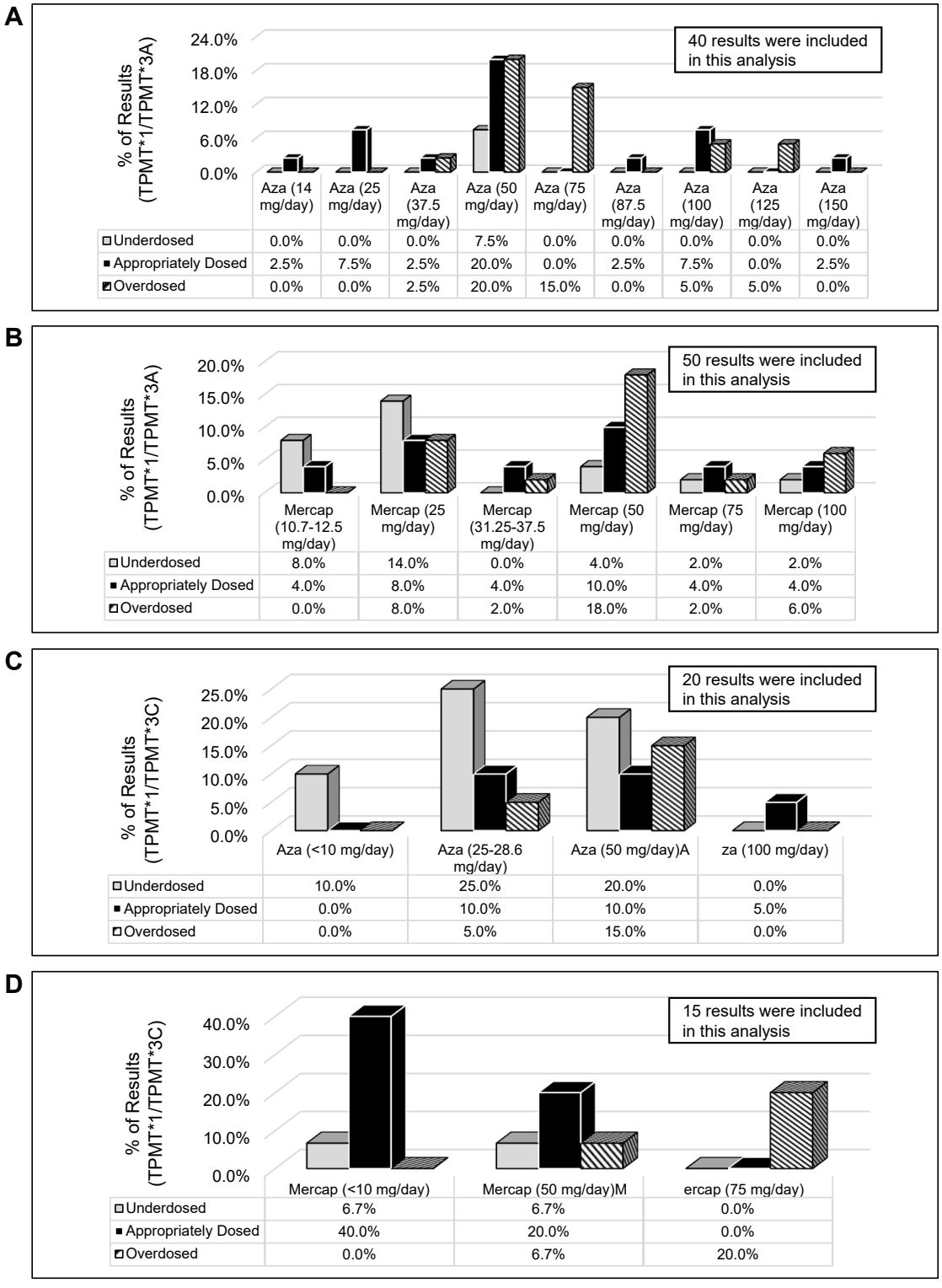


Figure 4 Genotype specific dosing distribution for heterozygous TPMT*1/TPMT*3A and TPMT*1/TPMT*3C individuals. (A) Distribution by percentage of TPMT*1/TPMT*3A individuals undergoing azathioprine treatment ranging between 14-150 mg/day. Doses higher than 150 mg/day or lower than 14 mg/day of azathioprine were not observed during this analysis. (B) Distribution by percentage of TPMT*1/TPMT*3A individuals undergoing mercaptopurine treatment ranging between 10.7-100 mg/day. Doses higher than 100 mg/day or lower than 10.7 mg/day of mercaptopurine were not observed during this analysis. (C) Distribution by percentage of TPMT*1/TPMT*3C individuals undergoing azathioprine treatment ranging between 7.1-100 mg/day. Doses higher than 100 mg/day or lower than 7.1 mg/day of azathioprine were not observed during this analysis. (D) Distribution by percentage of TPMT*1/TPMT*3C individuals undergoing mercaptopurine treatment ranging between 3.6-75 mg/day. Doses higher than 75 mg/day or lower than 3.6 mg/day of mercaptopurine were not observed during this analysis.

additional measurements are still needed to confirm these results. It is important to note that in all dosing situations analyzed, the need to use twice the concentration of Aza to 6-MP is consistent with previously published dosing recommendations [33].

The results suggesting that TPMT*1/TPMT*3C individuals benefit from a starting dose that is approximately half the dose needed by TPMT*1/TPMT*3A individuals to achieve appropriate dosing levels, prompted us to compare the distribution of 6-MMP analyte levels in our pool of results. Our goal was to determine if a difference in accumulated 6-MMP existed between TPMT*1/TPMT*3A and TPMT*1/TPMT*3C individuals, which might indicate a difference in overall TPMT activity. When results were evaluated, we observed that TPMT*1/TPMT*3A [Figure 5A] individuals had a wider distribution of 6-MMP values compared to individuals with the TPMT*1/TPMT*3C [Figure 5B] genotype. 74% of all 6-MMP quantities measured for TPMT*1/TPMT*3C individuals had 6-MMP concentrations of ≤100 pmol/8x108 RBCs, whereas 36% of all quantities measured for TPMT*1/TPMT*3A individuals had 6-MMP concentrations of ≤100 pmol/8x108 RBCs. In the case of TPMT*1/TPMT*3C individuals, no results were obtained with 6-MMP values between 200-1300 pmol/8x108 RBCs and only 4 values were observed to have values >1300 pmol/8x108 RBCs, 2 of which were observed in overdosed individuals and 2 were observed in patients with 6-TG values in the 300-400 pmol/8x108 RBCs range. The distribution observed in TPMT*1/TPMT*3A individuals was more evenly distributed supporting the observation that TPMT activity is likely higher in TPMT*1/TPMT*3A individuals, which is why they require twice the starting dose needed to reach "Appropriately Dosed" levels compared to TPMT*1/TPMT*3C individuals.

Through the course of this study only one individual with the TPMT*1/TPMT*2 genotype was identified and only two measurements were performed for this individual. Therefore, not enough information was acquired on this genotype group to draw conclusions or make dosing recommendations at this time.

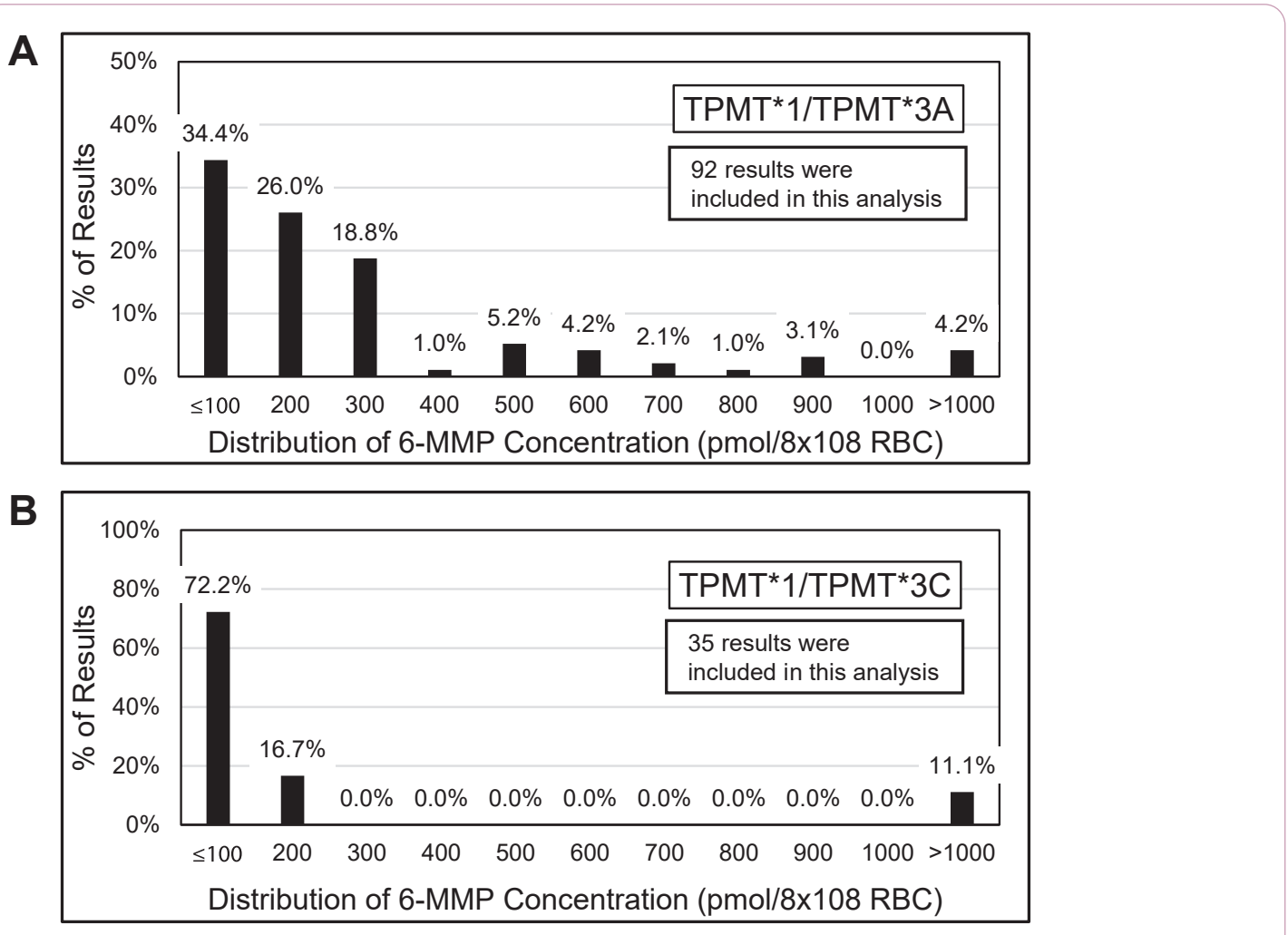


Figure 5 Distribution of 6-MMP results observed in heterozygous individuals. Bar charts illustrate the distribution of 6-MMP values quantified in results obtained for individuals encoding the genotypes TPMT*1/TPMT*3A (A) and TPMT*1/TPMT*3C (B). The percentage of results in the study with quantified 6-MMP values of ≤100 pmol/8x10^8 RBCs are included in the first bar, percentage of 6-MMP results with values of 101-200 pmol/8x10^8 RBCs are represented by the second bar and so on. The last measurement includes the percentage of results observed with 6-MMP quantified values of >1000 pmol/8x10^8 RBCs. This analysis was performed to evaluate TPMT activity in TPMT*1/TPMT*3A and TPMT*1/TPMT*3C heterozygous individuals.

Discussion

Taking a personalized approach to medicine is the future of health care. Harnessing genetic information to inform therapeutic approaches is paving the way to tailored medicine. In this study we employed a classical pharmacogenetic example, treatment of wild type and heterozygous individuals with purine analogs 6-MP and Aza, and added an additional layer of understanding to how heterozygous individuals carrying one of the two most prevalent single nucleotide polymorphisms TPMT*3A and TPMT*3C respond to treatment. This work was carried out using a diverse and expansive patient population of 946 individuals with known genotypes, of which 787 TPMT*1/TPMT*1, 50 TPMT*1/TPMT*3A, and 14 TPMT*1/TPMT*3C individuals had a known treatment and displayed analyte levels above the LLOQ. Patients with values below detectable levels were included in the population studies but were excluded from the dosing distribution analysis. Since many of the patients with values below detectable levels were tested prior to commencing treatment, after concluding treatment, or had documented instances of treatment noncompliance, inclusion of these individuals would have unfairly skewed the results.

Evaluating the results in a holistic manner, it is clear that achieving therapeutic levels is difficult due to many factors. Of the patient population studied, only 20.6% of TPMT*1/TPMT*1 individuals are "Appropriately Dosed", while 35.4% and 35.0% of TPMT*1/TPMT*3A and TPMT*1/TPMT*3C individuals, respectively, are "Appropriately Dosed". Factors that influence those low numbers include individual TPMT enzymatic activity, noncompliance, concomitant use of other medication, concerns due to increased susceptibility to infections as a result of using immunosuppressants, severe side effects resulting from overdosing and many other reasons [8, 33, 38-39]. Additionally, studies have shown that a lower therapeutic cut off (125 pmol/8x108 RBCs) is effective in treating patients with gastrointestinal disorders prescribed a combination therapy of thiopurines and anti-TNF agents, which may have contributed to a larger than expected group of patients with results in the "underdosed" category [40-41].

To improve compliance and prevent life threatening secondary effects associated with the use of purine analogs, it is essential to start at a safe lower dosage and titrate up. With this in mind, we evaluated the response of individuals with the three most prevalent genotypes, TPMT*1/TPMT*1, TPMT*1/TPMT*3A, and TPMT*1/TPMT*3C, to specific doses of Aza and 6-MP. Using the large amount of data acquired, we suggest genotype specific starting dosages, summarized in [Table 2]. The results presented clearly point to a lower overall TPMT activity in individuals carrying the TPMT*1/TPMT*3C genotype when compared to individuals with the TPMT*1/TPMT*3A genotype, which translates to requiring a lower dose of Aza and 6-MP during treatment. These results are consistent with earlier findings that suggest a lower overall activity in TPMT*1/TPMT*3C individuals [10]. Acquisition of future data will help to further refine the concentrations listed in this study. Furthermore, the information included here is presented as a starting point for treatment, weight-based calculation were not performed since studies have shown that weight-based dosing does not necessarily correlate with achieving therapeutic levels with thiopurine drugs [42-43].

Table 2: Genotype specific safe starting dose recommendations. Dosing recommendations were determined based on the results of the analysis presented in this study. These recommendations are suggested to improve compliance and minimize the potential secondary effects that are observed when overdosing occurs.

Genotype	Azathioprine (Aza)	Mercaptopurine (6-MP)
TPMT*1/TPMT*1	100 mg/day	50 mg/day
TPMT*1/TPMT*3A	50 mg/day	25 mg/day
TPMT*1/TPMT*3C	25 mg/day	12.5 mg/day

It is important to note that only 3.8% of wild type individuals treated with Aza and 8.8% of wild type individuals treated with 6-MP were undergoing treatment supplemented with allopurinol to achieve "Appropriately Dosed" levels. The larger scale use of allopurinol by clinicians in the future may prove beneficial in assisting more individuals shift from the "Shunter" and "Treatment Refractory" categories to the "Appropriately Dosed" category.

Lastly, this study lends credence to the need to measure TPMT activity prior to patients starting treatment. The data presented clearly indicates that within the larger TPMT*1/TPMT*1 genotype category, individuals required a wide range of doses to reach "Appropriately Dosed" levels. In the case of TPMT*1/TPMT*1 individuals treated with Aza, concentrations between 50-200 mg/day were needed. A four-fold difference between individual dosing needs is very large and demonstrates that a more direct measurement of TPMT activity would prove more useful in guiding treatment, than genotype alone. Spire-Vayron et al. (10) demonstrated that TPMT*1/TPMT*1 individuals can vary in TPMT activity between 10-50 U/ml RBC, which is equivalent to a 5-fold difference between the lowest to highest TPMT activity measured. This observation is consistent with the wide range of doses observed in this study, required to achieve therapeutic levels. In the three TPMT genotype categories studied >8.5% of results in each grouping were overdosed, increasing the risk of these patients to myelotoxicity and hepatoxicity. Measuring TPMT enzymatic activity prior to initiating treatment would provide physicians an extra layer of granularity in the decision-making process and would allow them to determine starting doses more accurately for patients with low to moderate TPMT activity. Knowing if a patient expresses high TPMT activity would allow physicians to treat patients with combination treatments, such as 6-MP and allopurinol, at the onset of treatment, greatly reducing the risk of hepatoxicity. Genotype alone does not allow physicians to identify patients with high TPMT activity but measuring enzymatic activity does. Understanding biochemically how a patient will respond prior to treatment will reduce treatment risk and lead to improved patient compliance.

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Conflict of Interest

The authors declare no conflict of interest in the preparation of this article.

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