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***TPMT* Genetic Variants Are Associated With Increased Rejection With Azathioprine Use In Heart Transplantation**

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Abstract

Objectives—Azathioprine (AZA) is an important immunosuppressant drug used in heart transplantation (HTX). Consensus guidelines recommend that patients with thiopurine S-methyltransferase (*TPMT*) genetic variants be started on lower AZA dose due to higher active metabolite levels and risk of adverse events. However, in vitro lymphocyte proliferation assays performed in subjects with inactive *TPMT* alleles have suggested that AZA use may result in decreased immunosuppressant efficacy as compared to wild type (WT) subjects. The objective of this study was therefore to determine the effect of *TPMT* genetic variation on AZA efficacy or prevention of rejection in HTX recipients treated with AZA.

Methods—We genotyped 93 HTX recipients treated with AZA and measured erythrocyte *TPMT* enzyme activity. Acute rejection was monitored for by routine endomyocardial biopsies.

Results—There were 83 WT and 10 heterozygote (HZ) HTX recipients. *TPMT* activity level was lower in HZ compared to WT (13.1 ± 2.8 vs. 21 ± 4.5 U/mL RBC, $p < 0.001$). Despite similar AZA dose, HZ developed severe rejection earlier ($p < 0.001$), and total rejection score was higher ($p = 0.02$) than WT. AZA was discontinued more frequently in HZ ($p = 0.01$) due to rejection. Incidence of leukopenia was similar between groups (40% vs. 43%, $p = 1.0$).

Conclusion—HTX recipients with *TPMT* genetic variant alleles who are treated with AZA develop acute rejection earlier, more frequently and of greater severity. These patients, despite having lower *TPMT* enzymatic activity, should be monitored carefully for possible increased risk of acute rejection.

Keywords

azathioprine; thiopurine S-methyltransferase; heart transplantation; rejection; toxicity; pharmacogenomics

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CONFLICTS OF INTEREST:

The authors have no conflicts of interests to disclose.

INTRODUCTION

Azathioprine (AZA) continues to be an important immunosuppressant drug used for prevention of rejection following solid organ transplantation due to lower costs and in patients with intolerance to mycophenolate mofetil (MMF) which can occur in 35–40% of heart transplant (HTX) recipients. [1] Clinical efficacy and development of side effects is dependent on AZA bioavailability, which is variable, in part, due to genetic variation in the drug's pharmacokinetic pathway. [2, 3]

Genetic variation in TPMT results in enhanced degradation of TPMT, leading to deficient TPMT enzyme activity. As a result, it could affect the “inactivation” or metabolism of AZA, potentially resulting in adverse effects such as leukopenia. Three nonsynonymous single nucleotide polymorphisms (SNPs) A719G, G460A and G238C account for 80–95% of the functional genetic variation observed in *TPMT*. [4] The variant alleles *TPMT**2 (G238C), *3B (G460A), *3C (A719G) and *3A (G460A and A719G) are present in approximately 10% of Caucasians. These SNPs alter the amino acid sequence of TPMT and ultimately lead to the formation of misfolded protein that is degraded by an ubiquitin-proteasome-mediated process. [5]

AZA can cause life-threatening myelosuppression and should be avoided altogether in patients homozygous for variant *TPMT* alleles. Heterozygotes (HZ) have one dysfunctional *TPMT* allele and below-normal TPMT activity. It has been recommended that these patients may need lower AZA dosage to achieve similar active metabolite levels as compared to wild-type (WT) patients. [6] Two previous studies in HTX have shown increased incidence of myelosuppression in recipients with low TPMT activity and *TPMT* polymorphisms respectively. [7, 8] Recent guidelines [9] recommend adjusting or decreasing the dosage when initiating AZA based on genetic variation. However, the impact of this genetic variation on the clinical “efficacy” of AZA in preventing rejection has not been explored, and the possibility exists that adjusting AZA dose due to genetic variation of *TPMT* could have important ramifications on this endpoint. We have demonstrated in a prior study that peripheral blood lymphocytes obtained from individuals who had inactive *TPMT* alleles when stimulated by mitogens appeared to be more “resistant” to the anti-proliferative effects of AZA and its metabolites. [10] The purpose of this study, therefore, was to investigate the relationship between TPMT enzymatic activity and genetic variation in *TPMT* with AZA clinical efficacy, especially prevention of rejection and safety in HTX recipients.

METHODS

Study population

A total of 93 HTX recipients (66 men and 27 women; mean age 49.4 years) who underwent HTX at Mayo Clinic, Rochester, MN and were treated initially with AZA were included in this study. All patients except one received initial induction therapy with a monoclonal antibody (muromonab-CD3, rabbit or equine antithymocyte globulin, or antilymphocyte globulin). Baseline immunosuppression was maintained with triple therapy that consisted of calcineurin inhibitor, AZA, and prednisone, which was subsequently tapered as per a standard protocol. Patients who received MMF at the outset, dual organ recipients, cardiac amyloidosis patients, and patients treated with additional drugs such as allopurinol, which are known to compete with AZA for metabolism, were excluded.

Clinical Data

This study was a retrospective database and medical record review for clinical data involving all patients who underwent cardiac transplantation and were treated with AZA.

Approval from the Mayo Clinic Institutional Review Board was obtained. Data from each patient was analyzed for the first 6 months following cardiac transplant, or until discontinuation of AZA.

TPMT genotyping

Genotyping was performed retrospectively by the Mayo Core Genotyping Laboratory for the following nonsynonymous SNPs: A719G, G460A and G238C. DNA was isolated from myocardial tissue or from blood samples that were collected and stored as part of a routine transplant research protocol. Genotyping was carried out using TaqMan (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions, using 10–20ng DNA. Primers and probes were from Assay-by-Design (Applied Biosystems). Following polymerase chain reaction amplification, end reactions were read on the ABI Prism 7900ht using Sequence Detection Software (Applied Biosystems). The quality value percentage is a quality metric that indicates the reliability of called genotypes generated by the SDS software. The quality value is calculated by using ABI's proprietary calling algorithm determining how well that sample fits into the cluster. Genotypes less than 95% are located further from their clusters and have a lower reliability. An electronic data file was generated which contained genotypes and the quality value. The expected minor allele frequency of *3C in Caucasians is 0.004; therefore all tracings of the Taqman assay results for the HZ patients were carefully reviewed. To ensure accuracy of genotyping results, multiple samples were analyzed in duplicate with 100% concordance.

TPMT activity assay

Prior to HTX, red blood cell (RBC) TPMT activity was assayed by the Mayo Biochemical Genetics Laboratory. The purpose of performing TPMT activity assays was to screen patients for low enzymatic activity who would have been excluded from receiving AZA as an immunosuppressant agent. Briefly, whole blood samples were obtained and red cells extracted and lysed. ³H-labeled S-adenosylmethionine and 6-MP were added to the lysates. The reaction was allowed to proceed for one hour. Transfer of the ³H-labeled S-methyl group to 6-MP is dependent on TPMT activity, and results in the formation of ³H-methyl-6-MP, which were then extracted and quantified by beta-scintillation counting. The results were expressed as nmol/h/ml of RBCs and one unit (U) of TPMT enzyme activity represents the formation of 1 nmol ³H-methyl-6-MP/hr/ml of packed erythrocytes at 37°C. The cutoffs in use in our laboratory were as follows: 15.1–26.4 U/mL RBC (normal), 6.3–15.0 U/mL RBC (intermediate TPMT activity), <6.3 U/mL RBC (low TPMT activity).

Toxicity

Leukopenia was defined as a leukocyte count less than $4.0 \times 10^9/L$, and severe leukopenia less than $3.0 \times 10^9/L$. The date the leukocyte counts first dropped below $4.0 \times 10^9/L$ and $3.0 \times 10^9/L$ were recorded and time to leukopenia was determined. The lowest leukocyte count in the study period while the patient was on AZA and the corresponding date were documented. Difference between pre-transplant baseline and lowest leukocyte counts while on AZA were recorded. For this analysis, in those patients whose lowest leukocyte count was higher than the baseline leukocyte count, the difference was recorded as zero. Hepatotoxicity was defined as an elevation of aminotransferases, bilirubin, and/or alkaline phosphatase greater than three times the upper limit of normal, prompting the supervising clinician to discontinue AZA with subsequent normalization of liver enzymes.

Rejection

Acute cellular rejection was monitored by regular surveillance endomyocardial biopsies as per routine protocol. All rejection scores for biopsies performed while on AZA therapy and/

or up to six months whichever occurred earlier were documented and analyzed. For analysis, all biopsy grades from biopsies performed prior to 2004 were adjusted per the 2004 International Society for Heart and Lung Transplantation (ISHLT) grading system. Severe rejection was defined as ISHLT grade 2R and above.

Total rejection score

Total rejection score (TRS) was determined for each patient during the 6-month study period or until discontinuation of AZA, whichever came first. TRS was calculated by dividing the sum of all rejection grades (1R=1, 2R=2, 3R=3) on biopsies by the total number of biopsies taken during the study period.

AZA dose change and discontinuation

AZA dose at baseline and following HTX, including all dosage adjustments were documented, together with the date and reason. AZA dose was adjusted as per WBC count. In those who discontinued AZA, the date and reason for discontinuation was recorded. AZA dosage at the end of the 6-month study period was documented for those who remained on AZA, while AZA dosage at time of discontinuation was documented for those who were discontinued from the medication prematurely.

Statistical analysis

T-tests were used to test for a difference in *TPMT* activity levels by genotype. Wilcoxon rank sum tests were used to compare AZA doses at transplant and 6 months post-transplant by genotype. Nonparametric Spearman correlations were used to test for associations of *TPMT* activity levels and AZA dose at transplant and 6 months post-transplant. The log-rank test was used to test for an association of genotype with the development of leukopenia and rejection within the first 6 months of HTX. Multivariable logistic regression analyses were used to evaluate whether association for acute rejection with *TPMT* activity and genotype was maintained after adjusting for other important covariates. Results of logistic regression analyses are reported as hazard ratios and 95% CI.

RESULTS

Baseline Characteristics

Genotyping resulted in the identification of 10 HZ *TPMT* variant and 83 *TPMT* WT patients. There were no homozygous *TPMT* variant patients. Baseline characteristics of the two groups are shown in Table 1. Mean age was similar between the two groups (49.4 ± 12.5 vs 49.8 ± 9.8 years, $p=0.766$). There was a trend towards there being more male patients in the WT group compared to HZ (75% vs 40%, $p=0.058$). Donor-recipient gender mismatches (17% vs 11%, $p=1.00$) and cytomegalovirus (CMV) mismatches (18% vs 20%, $p=1.00$) were similar between groups. The use of triple drug immunosuppression was similar in both groups.

TPMT Genotype and TPMT Activity

The frequencies of the genotypes were as follows: *TPMT**1/*1, $n=83$; *TPMT**1/*2, $n=1$; *TPMT**1/*3A, $n=5$; and *TPMT**1/*3C, $n=4$. The distribution of *TPMT* activity by genotype is illustrated in Figure 1. As expected, HZ had significantly lower *TPMT* activity levels as compared to WT (13.1 ± 2.8 vs. 21 ± 4.5 U/mL RBC, $p<0.001$). The concordance between HZ genotype and intermediate *TPMT* enzymatic activity level was 89%. There were no patients with low *TPMT* enzymatic activity.

AZA toxicity (leukopenia)

The data on AZA efficacy and toxicity are outlined in Table 2. There was no significant association between genotype and development of hepatotoxicity, leukopenia (Figure 2, A) or severe leukopenia (Figure 2, B) in the first 6 months of AZA therapy post-transplant despite the WT patients having a trend towards higher baseline leukocyte count than the HZ patients ($8.0 \pm 1.6 \times 10^9/L$ vs. $7.0 \pm 1.2 \times 10^9/L$, $p=0.06$). The lowest leukocyte counts achieved while on AZA during the entire study period were similar between the two groups ($4.6 \pm 2.1 \times 10^9/L$ vs. $4.7 \pm 1.7 \times 10^9/L$, $p=0.88$). Similar to HZ genotype, subjects with intermediate as compared to those with normal TPMT activity levels had similar incident rates of leukopenia ($p=0.78$) and severe leukopenia ($p=0.66$).

AZA efficacy (rejection)

Acute rejection occurred earlier in HZ versus WT patients (median 29 vs. 36 days, $p=0.046$) (Figure 3, A). Severe acute cellular rejection occurred more frequently (70% vs 15%, $p<0.001$) and earlier in the HZ group as compared to WT (median 68 vs. 180 days, $p<0.001$) (Figure 3, B). Mean TRS was significantly higher in the HZ versus WT patients (0.9 ± 0.6 vs 0.4 ± 0.3 , $p=0.02$). There were no significant differences in recipient sex, donor-recipient sex mismatch or CMV status mismatch (all risk factors for rejection) between the HZ and WT groups. Although there was a trend towards more females in the HZ group as compared to WT patients, sex difference (HR=0.98, $p=0.94$) and CMV status mismatch (HR=0.72, $p=0.21$) were not significant predictors of time to rejection.

We analyzed rejection outcomes in HTX recipients based on phenotype or TPMT activity (Figure 4). Patients with intermediate TPMT activity levels developed severe rejection earlier than patients with normal TPMT activity levels ($p<0.001$). There was also a trend of an increased total rejection score ($p=0.08$) in those with intermediate TPMT activity level. Multivariate analysis after adjusting for age, gender, and CMV mismatch status, identified both variant *TPMT* heterozygosity and intermediate TPMT activity level as independent predictors for rejection (HZ: HR=2.5, CI=1.2–5.1, $p=0.01$; intermediate TPMT activity: HR=2.0, CI=1.1–3.7, $p=0.02$) and severe rejection (HZ: HR=6.1, CI=2.1–18.2, $p=0.001$; intermediate TPMT activity: HR=4.68, CI=1.6–14.1, $p=0.006$).

AZA dose change and discontinuation

AZA dosing is described in Table 2. Mean starting AZA dose was similar in both groups (2.1 ± 0.4 mg/kg vs. 1.9 ± 0.5 mg/kg, $p=0.15$). Over the 6-month follow up period after HTX, HZ patients were significantly more likely to be discontinued from AZA as compared to WT (80% vs 34%, $p=0.01$). The dose at the time of AZA discontinuation or 6 months, whichever event was sooner, was not significantly different between the WT and HZ patients (1.9 ± 1.9 mg/kg and 1.6 ± 0.6 respectively, $p=0.24$). The reason for discontinuation was acute rejection in 7 (87.5%) discontinued HZ patients and 19 (67.9%) discontinued WT patients ($p=0.40$). The other reasons for AZA discontinuation were leukopenia (1 HZ, 5 WT) and hepatotoxicity (0 HZ, 4 WT). There were no patients who developed pancreatitis attributable to AZA, requiring AZA dose adjustment or discontinuation. There were also no significant differences between HZ and WT groups in the percentage of patients requiring AZA dose increase ($p=0.72$) or decrease ($p=0.74$) during the study period. Cyclosporine levels within the 6-month study period were similar between both groups (261 ± 77 ng/ml in WT and 305 ± 116 ng/ml in HZ, $p=0.229$), reflecting adequate immunosuppression with use of a calcineurin inhibitor. Cyclosporine levels at time of AZA discontinuation were also equivalent and therapeutic (mean 250–300 ng/ml) in the two groups, making sub-therapeutic cyclosporine levels an unlikely cause of acute rejection.

DISCUSSION

This is the only study in HTX examining the effect of TPMT genotype on the prevention of rejection with AZA use. This study presents completely novel and counter-intuitive findings of increased rejection associated with common genetic variation in *TPMT* with important clinical implications for AZA dosing when used in HTX, especially in the era of preemptive genotyping.

Only one prior study has examined the risk of AZA toxicity but not efficacy in HTX recipients with inactive *TPMT* alleles. [8] Due to the risk of toxicity in patients with inactive *TPMT* alleles, the recently published Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines [11] recommend starting AZA at 30–70% of target dose (e.g., 1–1.5 mg/kg/d) in disease states requiring that a “full-dose” be started at initiation, as would be the case for patients who undergo transplantation. These recommendations were based on a focused review of the literature, and a recent 2013 update [9] has not changed the current guidelines. We demonstrate that, despite starting AZA at the full-recommended dose in HZ HTX patients; myelosuppression was not observed but instead, paradoxically, rejection occurred more frequently and with greater severity in HZ patients as compared to WT.

In HTX, the vast majority of rejection episodes occur within the first 3 months after transplant, [12] and 80% of HZ patients in our study had switched to MMF, suggesting 6-months was an adequate follow-up period. Preemptive identification of *TPMT* HZ or homozygote patients is recommended prior to AZA use [13] since dose adjustment based on genotype has been shown to minimize toxicity without compromising clinical efficacy in various disease states including rheumatic diseases, acute lymphocytic leukemia and inflammatory bowel disease. [6, 14–17] However, clinical efficacy data on the use of AZA as an anti-rejection or immunosuppressant agent in transplantation is limited. Kurzwski et al [18] demonstrated that the number of HZ renal transplant patients (n=2, 15%) who experienced rejection were similar to WT (n=20, 20%). Amongst the 8 HZ renal transplant recipients in a study by Song et al, [19] there was a trend towards increased rejection in HZ (25%) as compared to WT patients (21.9%). In both studies, the starting AZA dose was not adjusted based on genotype, so the implications for a reduced AZA dose are unknown. These studies also do not provide details, such as number of biopsies performed and severity of rejection episodes in the HZ and WT groups to allow for comprehensive determination of clinical efficacy. We demonstrate that HZ HTX patients experience far greater number and severity of rejection episodes requiring eventual AZA discontinuation as compared to WT patients. The increased risk for rejection in HZ patients occurred despite similar immunosuppressant regimens and full recommended starting dose of AZA. This increased risk persisted despite adjusting for gender and other risk factors for rejection like donor-recipient sex mismatch and CMV status in the HZ patients.

The relative discordance between genetic variants assessed for in this study and TPMT activity may be attributable to the fact that genotyping was performed for the three most common *TPMT* variants that account for over 80% of the individuals with low or intermediate TPMT activity. [20] Subjects with low or intermediate TPMT activity may have other rare *TPMT* variants or TPMT activity may also be affected by polymorphisms in other genes such as *PACSLN2*. [21–24]. We therefore also analyzed rejection outcomes in HTX recipients based on phenotype or TPMT activity. We demonstrated that patients with intermediate TPMT activity levels were at increased risk for severe rejection and intermediate TPMT activity adjusted for age, sex and CMV mismatch status continues to remain as a significant predictor of rejection.

The increased incidence of rejection in *TPMT* HZ patients receiving AZA may initially seem counterintuitive since HZ patients typically have higher active AZA major metabolite TGN levels. One of the limitations of our study was that we did not measure these metabolite levels since they were not routinely used to guide AZA dosing in our clinical practice. However, HZ patients may have lower levels of methylmercaptapurine nucleotide (MeMPN), an AZA metabolite, since *TPMT* plays a role in the formation of MeMPN by methylating thioinosine monophosphate. MeMPN has immunosuppressant activity by inhibiting purine de novo synthesis and blocking proliferation of lymphocytes. [25] Hence, a lower level of MeMPN may result in an increased risk of rejection. Our laboratory has also demonstrated during *in vitro* experiments that human peripheral blood lymphocytes obtained from individuals who have inactive *TPMT* alleles when stimulated by mitogens have higher 50% effective dose (ED₅₀) and inhibition constant (K_i) values for inhibition of (³H)thymidine incorporation into DNA by 6-MP [10] Therefore in subjects with *TPMT* genetic variants, the peripheral lymphocyte, an important “effector” of acute rejection, may be relatively resistant to the immunosuppressant actions of AZA, which may explain the increase in rejection episodes we observed in our patient population. The concept of genetic variation affecting drug response is not new and has been observed with beta-adrenergic receptor genetic variation and bucindolol use in heart failure in the Beta-Blocker Evaluation of Survival Trial (BEST) trial wherein patients with the Arg389 genetic variant had a statistically significant 38% reduction in mortality whereas Gly389 carriers had no significant change in mortality with bucindolol treatment. [26] Our findings raise the important question as to whether increased rejection with AZA use observed in the multicenter trials comparing AZA and MMF in HTX may have been due in part to *TPMT* genetic variants.

The clinical evidence linking myelosuppression with *TPMT* activity and genotype is better established than that for clinical efficacy in transplantation. *TPMT* HZ status and intermediate RBC *TPMT* activity have been shown to be independently predictive of increased likelihood of toxicity, with one meta-analysis stating an odds ratio of 4 for developing leukopenia in these patients. [27] Most of the transplant studies, however, have been performed in patients who received renal or hepatic transplants. Only one small study in HTX patients has examined the relationship between *TPMT* heterozygosity and toxicity. Sebbag et al [8] prospectively analyzed 30 patients treated with AZA following HTX, 4 of whom were *TPMT* HZ. Amongst the 4 HZ patients, 2 developed agranulocytosis and 2 had a 40% drop in neutrophil count which was reversed upon discontinuation of AZA. [8] Our study demonstrates that, despite starting the full-recommended dose of AZA in both WT and HZ HTX recipients, leukopenia occurred with similar frequency in both groups. Possible explanations for HZ patients not having higher rates of leukopenia versus WT patients are a higher baseline WBC count and early AZA discontinuation due to development of rejection. The reason for a trend towards higher baseline WBC count in the HZ patients in our study is unknown and this phenomenon has not been observed in other clinical trials or studies. Our finding that *TPMT* heterozygotes are not at an increased risk for myelosuppression has also been observed in patients with acute lymphoblastic leukemia and inflammatory bowel disease treated with AZA and 6-mercaptopurine. [28–30]. Leukopenia typically occurs within days after initiation of AZA in homozygote patients, however in HZ patients it can occur after several months. [31] Median time to AZA discontinuation, which was predominantly due to rejection, in the 6 HZ patients who did not develop leukopenia was 40 days, which is less than the median time to development of leukopenia in the WT group (58 days). Furthermore, leukopenia may not have been observed because AZA dose was adjusted based on WBC count, although this adjustment was made in both groups and AZA dose was similar at time of discontinuation. Our myelosuppression results are consistent with those of the TARGET (*TPMT*: Azathioprine Response to Genotyping and Enzyme Testing) study, a prospective randomized controlled clinical trial evaluating the effect of a

TPMT genotyping strategy to dose AZA in patients treated for non-transplant related inflammatory diseases, a study that demonstrated no difference in myelosuppression or AZA discontinuation due to adverse drug reactions in *TPMT* HZ patients as compared to WT. [32]

Although current literature supports the concept that homozygote patients with *TPMT* variant alleles are at risk for toxicity with AZA therapy, our results suggest that *TPMT* HZ HTX recipients are at an increased risk for rejection without preemptive AZA dose adjustments. In HZ patients, dose reductions, if instituted with preemptive genotyping as recommended in current CPIC guidelines [11], should be accompanied by close monitoring for clinical efficacy or lack thereof (rejection). This study should be considered preliminary and further replication is required to confirm our findings.

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Figure 1.
Distribution of erythrocyte TPMT activity by TPMT genotype

Figure 2.
Time to onset of leukopenia (A) and severe leukopenia (B) in TPMT heterozygotes compared to wild type

Figure 3.
Time to onset of rejection (A) and severe rejection (B) in TPMT heterozygotes compared to wild type

Figure 4. Time to onset of rejection (A) and severe rejection (B) in patients with intermediate versus normal TPMT activity levels

Table 1

Baseline Characteristics of TPMT WT and HZ patients treated with AZA

	TPMT WT (<i>n</i> =83)	TPMT HZ (<i>n</i> =10)	p-value	All (<i>n</i> =93)
Recipient Age, yrs	49.4 ± 12.5	49.8 ± 9.8	0.766	49.4 ± 12.2
Donor Age, yrs	33.6 ± 13.5	33.9 ± 15.4	0.985	33.7 ± 13.6
Male Recipient Sex, <i>n</i> (%)	62 (74.7)	4 (40.0)	0.058	66 (71.0)
Male Donor Sex, <i>n</i> (%)	59 (72.8)	5 (55.6)	0.275	64 (71.1)
Sex mismatch, <i>n</i> (%)	14 (17.3)	1 (11.1)	1.000	15 (16.7)
Positive Recipient CMV status, <i>n</i> (%)	43 (53.1)	8 (80.0)	0.176	51 (56.0)
Positive Donor CMV status, <i>n</i> (%)	50 (66.7)	6 (75.0)	1.000	56 (67.5)
CMV status mismatch, <i>n</i> (%)	15 (18.3)	2 (20.0)	1.000	17 (18.5)
Caucasian race, <i>n</i> (%)	81 (97.6)	9 (90.0)	0.292	90 (96.8)
Weight, kg	77.5 ± 18.9	72.4 ± 15.3	0.556	77.0 ± 18.6
Ischemic cardiomyopathy, <i>n</i> (%)	28 (33.7)	4 (40.0)	0.732	4 (34.4)
TPMT activity (mean U/mL RBC)	21.0 ± 4.5	13.1 ± 2.8	<0.001	20.2 ± 5.0
Cyclosporine, <i>n</i> (%)	77 (92.8)	9 (90)	0.562	86 (92.5)
Tacrolimus, <i>n</i> (%)	6 (7.2)	1 (10)	0.562	7 (7.5)
Muromonab-CD3, <i>n</i> (%)	75 (90.4)	10 (100)	0.592	85 (91.4)
Anti-thymocyte globulin, <i>n</i> (%)	3 (3.6)	0 (0)	1.000	3 (3.2)
Anti-lymphocyte globulin, <i>n</i> (%)	4 (4.8)	0 (0)	1.000	4 (4.3)

Abbreviations: TPMT, thiopurine S-methyltransferase; WT, wild-type; HZ, heterozygote; AZA, azathioprine; CMV, cytomegalovirus; RBC, red blood cell

Table 2

Immunosuppression regimen, rejection and toxicity based on TPMT genotype

	WT (n=83)	HZ (n=10)	p-value	All (n=93)
Initial AZA dose, mg/kg	2.14 ± 0.41	1.91 ± 0.47	0.145	2.12 ± 0.42
AZA dose at time of AZA discontinuation (or 6 months), mg/kg	1.87 ± 1.92	1.61 ± 0.60	0.243	1.84 ± 0.61
AZA discontinuation, n (%)	28 (33.7%)	8 (80.0%)	0.012	36 (38.7%)
AZA dose increase, n (%)	26 (31.3%)	2 (20.0%)	0.718	28 (30.1%)
AZA dose decrease, n (%)	33 (39.8%)	5 (50%)	0.735	38 (40.9%)
Mean Cyclosporine level while on AZA, ng/mL	261.1 ± 77.0	304.6 ± 116.0	0.229	266.0 ± 82.6
Mean Cyclosporine level at time of AZA discontinuation, ng/mL	293.0 ± 97.2	298.0 ± 103.6	1.000	294.0 ± 96.7
Number of biopsies	7.87 ± 3.17	5.80 ± 4.96	0.182	7.7 ± 3.4
Total rejection sum	3.33 ± 2.33	3.50 ± 2.37	0.858	3.4 ± 2.3
Total rejection score	0.43 ± 0.31	0.87 ± 0.64	0.022	0.5 ± 0.4
Any rejection, n (%)	72 (86.8%)	10 (100%)	0.601	82 (88.2%)
Median time to rejection, days	36	29	0.046	35
Severe rejection, n (%)	12 (14.6%)	7 (70.0%)	<0.001	19 (20.7%)
Median time to severe rejection, days	180	68	<0.001	180
Hepatotoxicity, n (%)	7 (8.4%)	0 (0%)	1.000	7 (7.5%)
Leukopenia ($< 4.0 \times 10^9/L$), n (%)	36 (43.4%)	4 (40.0%)	1.000	40 (43.0%)
Severe leukopenia ($< 3.0 \times 10^9/L$), n (%)	19 (22.9%)	2 (20.0%)	1.000	21 (22.6%)
Lowest WBC count ($\times 10^9/L$)	4.62 ± 2.1	4.65 ± 1.7	0.877	4.6 ± 2.1
Baseline WBC count ($\times 10^9/L$)	7.01 ± 1.2	8.02 ± 1.6	0.055	7.1 ± 1.3

Abbreviations: AZA, azathioprine; WT, wild-type; HZ, heterozygote; WBC, white blood cell