Mercaptopurine Therapy Intolerance and Heterozygosity at the Thiopurine S-Methyltransferase Gene Locus


Background: Patients with acute lymphoblastic leukemia are often treated with 6-mercaptopurine, and those with homozygous deficiency in thiopurine S-methyltransferase (TPMT) enzyme activity have an extreme sensitivity to this drug as a result of the accumulation of higher cellular concentrations of thioguanine nucleotides. We studied the metabolism, dose requirements, and tolerance of 6-mercaptopurine among patients with different TPMT phenotypes. Methods: We compared, by use of statistical modeling, 6-mercaptopurine pharmacology and tolerance in 180 patients who achieved remission on St. Jude Children’s Research Hospital Protocol Total XII composed of weekly methotrexate (40 mg/m²) and daily oral 6-mercaptopurine (75 mg/m²) given for 2.5 years, interrupted every 6 weeks during the first year for treatment with either high-dose methotrexate or teniposide plus cytarabine. Statistical tests were two-sided. Results: Erythrocyte concentrations of thioguanine nucleotides (pmol/8 × 10⁸ erythrocytes) were inversely related to TPMT enzyme activity (P < .01), with averages (± standard deviations) of 417 (±179), 963 (±752), and 3565 (±1282) in TPMT homozygous wild-type (n = 161), heterozygous (n = 17), and homozygous-deficient (n = 2) patients, respectively. There was complete concordance between TPMT genotype and phenotype in a subset of 28 patients for whom TPMT genotype was determined. There were no sex differences in thioguanine nucleotide concentrations (P = .24), TPMT enzyme activity (P = .22), or average weekly prescribed dose of 6-mercaptopurine (P = .49). The cumulative incidence of 6-mercaptopurine dose reductions due to toxicity was highest among patients homozygous for mutant TPMT (100%), intermediate among heterozygous patients (35%), and lowest among wild-type patients (7%) (P < .001), with average (± standard deviation) final weekly 6-mercaptopurine doses of 72 (±60), 449 (±160), and 528 (±90) mg/m², respectively. Lowering doses of 6-mercaptopurine in TPMT heterozygotes and in deficient patients allowed administration of full protocol doses of other chemotherapy while maintaining high thioguanine nucleotide concentrations. Conclusion: We conclude that genetic polymorphism in TPMT is an important determinant of mercaptopurine toxicity, even among patients who are heterozygous for this trait. [J Natl Cancer Inst 1999;91:2001–8]
randomly assigned to receive “pulse” therapy with high-dose methotrexate at a dose of 1.5 g/m² over a 24-hour period in the conventional group and individualized to achieve a target area-under-the-concentration - time (AUC) curve in the targeted group alternating with teniposide plus cytarabine (see (12) and Fig. 1 for details) every 6 weeks, with doses based on either body surface area or doses individualized on the basis of pharmacokinetic parameters. During other weeks, patients received weekly methotrexate at a dose of 40 mg/m² (intravenously or intramuscularly) and daily oral 6-mercaptopurine at a dose of 75 mg/m², for a total of 2.5 years. Complete blood cell counts were obtained weekly. Chemotherapy was given every week, provided that the absolute neutrophil count was greater than 300 cells/µL and that the patient did not exhibit other complications, such as mucositis, fever, or hepatotoxicity. If toxic effects or neutropenia in any given week precluded administration of chemotherapy, the scheduled therapy with high-dose methotrexate or teniposide plus cytarabine was delayed until the patient recovered, whereas the scheduled low-dose methotrexate plus 6-mercaptopurine was omitted altogether.

**Erythrocyte Thiopurine Metabolites**

Erythrocyte concentrations (pmol/L x 10⁶ erythrocytes) of thioguanine nucleotides and of thioguaninosine monophosphate were measured by hydrolyzing erythrocyte lysates with acid and heat to the respective thioguanine and 6-mercaptopurine bases, as previously described (13). Methylated metabolites of methylthioinosine monophosphate were measured by hydrolyzing to methyl-6-mercaptopurine in a subset of patients in a separate high-pressure liquid chromatography (HPLC) assay (14). Patients were scheduled to have thiopurine metabolite concentrations measured in erythrocytes at weeks 7, 31, 55, 82, 106, and 120 of continuation therapy. At each of these times, the treatment protocol specified that patients should have received daily 6-mercaptopurine for at least the prior 5 weeks. Patients were instructed to take their 6-mercaptopurine on an empty stomach in the evening, and all samples were obtained at least 8 hours after the preceding 6-mercaptopurine dose. Because of acute toxicity, noncompliance, or other unusual reasons (e.g., misunderstanding directions, vacations, etc.), some patients might not have received 6-mercaptopurine daily during this time period. Lack of dosing was not a reason for not obtaining a sample at the scheduled time. For each sample, a research nurse reviewed with the patient and his/her guardian the dosing history of 6-mercaptopurine for the preceding 6 weeks and documented her subjective assessment of compliance, the time of day that the child had taken 6-mercaptopurine in the prior dosing interval, and the reason for obtaining the erythrocyte sample (specified by the protocol or for suspected toxicity or noncompliance). Ninety-seven percent of the samples were obtained as specified by the protocol, with 3% for toxicity or suspected noncompliance. Only samples obtained at times specified by the protocol were included in statistical analyses to assess pharmacologic measures versus toxicity or sex; all samples were eligible for inclusion for the assessment of thioguanine nucleotide concentrations versus compliance.

**Plasma AUC-Versus-Time Curves**

Plasma AUCs for methotrexate, cytarabine, and teniposide were measured in patients for every course of pulse chemotherapy, as previously described (12).

**TPMT Phenotype and Genotype**

Erythrocyte TPMT activity was measured by use of blood collected in heparinized tubes, as previously described (11). Erythrocyte TPMT activity was measured greater than or equal to 90 days following the last erythrocyte transplanted in 109 patients during their continuation therapy (1–843 days following achievement of complete remission) and in 45 patients after completion of continuation therapy (499–1602 days following achievement of complete remission). If a patient had TPMT measured while on therapy, the lowest value was used to assign phenotype as follows: less than or equal to 5 U/mL of packed erythrocytes, homozygous mutant; greater than 5 but less than 13.5 U/mL of packed erythrocytes, heterozygotes; and greater than or equal to 13.5 U/mL of packed erythrocytes, wild-type. If the TPMT was measured only after completion of continuation therapy, the lowest value was used to assign phenotype as follows: less than or equal to 5 U/mL of packed erythrocytes, homozygous mutant; greater than 5 but less than 10.2 U/mL of packed erythrocytes, heterozygous; and greater than or equal to 10.2 U/mL of packed erythrocytes, homozygous wild-type. If patients did not have TPMT activity measured during or after completion of therapy but had erythrocyte thioguanine nucleotide concentrations below the 90th percentile for maximum thioguanine nucleotides for the entire group (1120 pmol/L x 10⁶ erythrocytes), they were considered to be wild-type; above that level, they were considered to have been heterozygous. All 26 children who had no measures of TPMT activity were classified as wild-type, whereas all of the children classified as heterozygotes or mutant had their TPMT values measured to substantiate that classification. The average thioguanine nucleotides among the children assigned to the wild-type group on the basis of low thioguanine nucleotides were not different from the average thioguanine nucleotides in patients who were classified as wild-type on the basis of measured TPMT activity (P = .857). Of the 182 patients who entered remission, either thioguanine nucleotides or TPMT activity was evaluable in 180 patients (for purposes of assigning phenotype). TPMT genotype was determined in a subset of patients with each phenotype by use of leukocyte DNA and polymerase chain reaction-based methods specific for the TPMT *2, *3A, *3B, and *5C mutant alleles, as previously described (15).
Doses of Continuation Chemotherapy

A patient-specific treatment calendar, specifying doses of chemotherapy for all 120 weeks of continuation therapy, was kept in the patient’s medical record and updated regularly by clinical and research staff. All pulses of high-dose methotrexate, teniposide, and cytarabine were administered at St. Jude Children’s Research Hospital. The exact doses and reasons for any deviations from the planned protocol therapy for every dose of every antileukemic medication were compiled into an institutional database. One of the patients with extreme intolerance to continuation chemotherapy was identified to be homozygous deficient for TPMT (3). A drastic dose reduction (from 75 mg/m² per day to 10 mg/m² given 3 days per week) resulted in excellent tolerance and allowed administration of full doses of the remainder of continuation therapy. From that point forward, if clinicians asked for a pharmacokinetic consultation on the TPMT status and thioguanine nucleotide concentrations of a patient experiencing unusual toxicity to therapy, consultations on thiopurine status were provided. Doses of 6-mercaptopurine were decreased gradually in patients with likely heterozygous status until reaching a dose that resulted in the desired degree of leukopenia (<4000 cells/µL but absolute neutrophil count >300 cells/µL) and allowed full doses of other antileukemic agents. Doses were decreased only in those patients experiencing myelosuppression. In addition, the 6-mercaptopurine dose was increased after week 60 of continuation therapy in case of persistently high leukocyte counts (>4000/µL and absolute neutrophil counts >1500/µL for 4 consecutive weeks). No dose changes in weekly methotrexate were dictated by the protocol.

Evaluation of Toxic Effects

For each week that therapy was withheld because of toxicity, the primary reason was documented (e.g., neutropenia, hepatotoxicity, thrombocytopenia, etc.). Hospitalizations for fever or infection were also considered to be toxic effects of therapy. If the 6-mercaptopurine dose was reduced from the protocol-specified dose of 525 mg/m² per week (75 mg/m² per day), toxicity was assessed only for the time period up until a dose reduction was required.

Statistical Analysis

The interpatient coefficient of variation for average values of the 6-mercaptopurine metabolites (thioguanine nucleotides, thioinosine monophosphates, and methlythioinosine monophosphate) as well as TPMT activity was computed as the ratio of the standard deviation (SD) to the mean from all contributed measurements and expressed as a percentage. The average intrapatient coefficient of variation was computed as the ratio of the SD to the mean for all measurements contributed by an individual patient and then averaged across all patients and expressed as a percentage.

Generalized estimating equations for longitudinal correlated binary data (16) were used to test for differences between heterozygotes and homozygous wild-types with respect to the incidence of primary toxic effects, including missed doses of mercaptopurine, hospitalizations for fever and neutropenia, neutropenia, or episodes of hepatotoxicity or thrombocytopenia. For each of the end points, each week of continuation therapy was censored with a binary variable representing the occurrence of the toxicity of interest.

Differences in doses and areas under the time–concentration curves for high-dose methotrexate, teniposide, and cytarabine among the three TPMT phenotypes were modeled and compared with the method of Diggle et al. (16) to account for repeated measures with missing data. Each of the models included treatment arm as a covariate. The same model was used to test for differences in thioguanine nucleotide levels between the sexes, between compliant and non-compliant patients, and between patients whose TPMT phenotype was assigned on the basis of thioguanine nucleotide levels and those assigned on the basis of measured TPMT activity and to test for differences in 6-mercaptopurine doses and TPMT activity between the sexes. Fisher’s exact test was used to test for an association of TPMT phenotype with sex.

The cumulative incidences of patients who required 6-mercaptopurine dose adjustments to prevent toxicity were estimated for each of the three TPMT phenotypes by the method of Kalbfleisch and Prentice (17) and were compared with Gray’s test (18). All statistical tests were two-sided.

RESULTS

Of the 188 patients enrolled on protocol Total XII, 182 achieved a complete remission; their demographic characteristics have been described previously (12). Erythrocyte concentrations of thioguanine nucleotides, thioinosine monophosphate, methylthioinosine monophosphate, and TPMT activity are shown in Table 1. There was substantial variability in erythrocyte concentrations of these active 6-mercaptopurine metabolites, with greater variability across the entire population (interpatient coefficient of variation = 78.0% for thioguanine nucleotides) than within individual patients (average intrapatient coefficient of variation = 37.9%).

TPMT phenotype was assigned for 180 of 182 patients who achieved a complete remission based on TPMT activity in 154 patients and on erythrocyte thioguanine nucleotides in 26 additional patients and on erythrocyte thioguanine nucleotides in 26 additional patients.

Table 1. Thiopurine pharmacologic variables among 180 patients with acute lymphoblastic leukemia

<table>
<thead>
<tr>
<th></th>
<th>No. of measures</th>
<th>No. of patients</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Interpatient coefficient of variation, %</th>
<th>Average intrapatient coefficient of variation, %</th>
<th>No., for intrapatient coefficient of variation</th>
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<tr>
<td>Average thioguanine nucleotides*,†</td>
<td>673</td>
<td>176</td>
<td>477</td>
<td>372</td>
<td>78.0</td>
<td>37.9</td>
<td>151</td>
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<td>Maximum thioguanine nucleotides*,‡</td>
<td>180</td>
<td>180</td>
<td>705</td>
<td>509</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Average thioinosine monophosphate*,†</td>
<td>673</td>
<td>176</td>
<td>91</td>
<td>84</td>
<td>92.2</td>
<td>79.2</td>
<td>137</td>
</tr>
<tr>
<td>Maximum thioinosine monophosphate*,‡</td>
<td>180</td>
<td>180</td>
<td>143</td>
<td>98</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Average methylthioinosine monophosphate*,†</td>
<td>118</td>
<td>76</td>
<td>16249</td>
<td>12820</td>
<td>78.9</td>
<td>46.7</td>
<td>34</td>
</tr>
<tr>
<td>Maximum methylthioinosine monophosphate*,‡</td>
<td>86</td>
<td>88</td>
<td>17253</td>
<td>12598</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>Thiopurine S-methyltransferase activity§</td>
<td></td>
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<td>On therapy</td>
<td>160</td>
<td>109</td>
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<td>11.9</td>
<td>51</td>
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<tr>
<td>Off therapy</td>
<td>107</td>
<td>88</td>
<td>16.9</td>
<td>6.1</td>
<td>36.2</td>
<td>17.8</td>
<td>19</td>
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</tbody>
</table>

*For those variables for which more than one measurement was taken per patient, the median and range of all possible values are indicated. The average value is the average of all measures made following a dose of 65–85 mg/m² for at least 12 of the prior 14 days, except for the two deficient patients, for whom the first measures are included for each (one at 75 mg/m² per day for 7 days and one at 50 mg/m² per day for 3 days per week). For interpatient coefficient of variation, the number of patients is identical to that in column 3.
†pmol/8 × 10² erythrocytes.
‡The maximum of all measures, regardless of dose.
§Units per milliliters of packed erythrocytes; on-therapy values were measured during the 120 weeks of continuation therapy, and off-therapy values were measured at least 2 months after stopping continuation therapy. N/A = not applicable.
tional patients who did not have TPMT activity measured. Of these 180 patients, two patients were TPMT homozygous-deficient phenotypes, 17 were heterozygous phenotype, and 161 were homozygous wild-type phenotype. Two patients did not have TPMT phenotype assigned because they had neither TPMT activity nor erythrocyte thioguanine nucleotides measured. TPMT genotype was determined in 28 patients representative of each phenotype and was in complete concordance with assigned phenotype: 18 homozygous wild-type (all TPMT*1/*1), eight heterozygous (all TPMT*1/*3A), and both homozygous-mutant individuals (one *2/*2 and one *3A/*2).

Fig. 2 is a frequency histogram showing the average (within a patient) of all thioguanine nucleotide concentrations measured immediately after a period during which patients received at least 65 mg/m² per day for 12 of the preceding 14 days. Erythrocyte concentrations of thioguanine nucleotides were higher and those of methylthioinosine monophosphate were lower among TPMT homozygous-deficient patients, while metabolite concentrations were intermediate among heterozygotes (Fig. 3). There was a statistically significant (P<0.01) inverse relationship between concentration of thioguanine nucleotides (Fig. 4) and TPMT, with an average (SD) concentration (pmol/8 × 10⁸ erythrocytes) of 417 (179), 963 (752), and 3565 (1282) in TPMT homozygous wild-type (n = 161), heterozygous (n = 17), and homozygous-deficient (n = 2) patients, respectively. There was also a positive relationship between thioinosine monophosphate and TPMT (P<0.01).

Fig. 5 shows the frequency of administration of 6-mercaptopurine or the reasons for withholding 6-mercaptopurine during every eligible week of continuation therapy for each patient in the three TPMT phenotype groups. TPMT-deficient patients tolerated full-dose 6-mercaptopurine only 7% of weeks, heterozygotes 65% of weeks, and homozygous wild-type 84% of weeks over the 2.5 years of therapy. Likewise, the percentage of weeks during which 6-mercaptopurine dose was decreased to prevent toxicity was 2%, 16%, and 76% among wild-type, heterozygous, and homozygous-mutant phenotypes, respectively. Heterozygotes were significantly more likely to miss weeks of 6-mercaptopurine than homozygous wild-type patients for any reason (P = 0.003) or specifically because of neutropenia (P = 0.007), although they were not more likely to be hospitalized for fever or infection (P = 0.128) (Table 2). Furthermore, the percentage of patients for whom the maximum sustainable 6-mercaptopurine dose was less than, equal to, or greater than the protocol starting dose of 525 mg/m² per week was 4%, 83%, and 13% for TPMT wild-type; 19%, 75%, and 6% for heterozygous; and 100%, 0%, and 0% for homozygous-mutant TPMT patients, respectively. Fig. 6 shows the cumulative incidence of a 6-mercaptopurine dose decrease due to toxicity for patients with each of the three TPMT phenotypes; all (100%) homozygous-deficient patients.
deficient, 35% of heterozygous, and 7% of homozygous wild-type patients eventually required a 6-mercaptopurine dose decrease ($P < .001$), with an average (SD) of final weekly 6-mercaptopurine doses of 72 (60), 449 (160), and 528 (90) mg/m², respectively. More important, although patients with a genetic defect in TPMT required reduced doses of 6-mercaptopurine, their doses of low-dose methotrexate, high-dose methotrexate, teniposide, and cytarabine were not lower than those without a defect (data not shown).

Each time blood was obtained for measurement of thiopurine metabolites, a research nurse subjectively assessed whether the patient was compliant or noncompliant with 6-mercaptopurine therapy. Eight patients were deemed noncompliant on 13 occasions; thioguanine nucleotides were lower during these episodes of presumed noncompliance compared with the 638 thioguanine nucleotide measurements at times when children were deemed compliant (mean ± SD of 278 ± 185 pmol/8 × 10⁸ erythrocytes versus 482 ± 329 pmol/8 × 10⁸ erythrocytes; $P = .025$). Thioguanine nucleotides were undetectable in only three children at times when they had been prescribed full-dose 6-mercaptopurine for the preceding 2 weeks, one of whom was deemed noncompliant by subjective assessment. Compliance was strongly reinforced with these children and parents following documentation of undetectable thioguanine nucleotides; for both children who subsequently had repeat measurements, thioguanine nucleotides were readily detectable on follow-up. All three of these children remain in continuous complete remission.

Neither thioguanine nucleotides ($P = .24$) nor prescribed 6-mercaptopurine dose ($P = .49$; not shown) differed between boys and girls. The average (SD) TPMT value did not differ

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**Fig. 4.** Maximum red blood cell (RBC) thioguanine nucleotides (TGNs) versus minimum RBC thiopurine S-methyltransferase (TPMT) activity in units per milliliter of packed RBCs (PRBCs). Accounting for all data and multiple observations, the relationship is statistically significant ($P < .01$; see the “Results” section). Best fit line was estimated by use of the distance-weighted, least-squares procedure as implemented in Statistica (StatSoft Inc., Tulsa, OK).

**Fig. 5.** Descriptors for all weeks of continuation therapy, during which 6-mercaptopurine (6MP) should have been given (through week 120 or time of censoring, if applicable) among the 161 patients (pts) who were homozygous wild-type, the 17 who were heterozygous, or the two who were homozygous deficient for thiopurine S-methyltransferase.
statistically \((P = .22)\) in boys \((17.1 \pm 5.5 \text{ U/mL of packed erythrocytes})\) versus girls \((18.2 \pm 5.2 \text{ U/mL of packed erythrocytes})\), nor was there a statistically significant difference in the proportion of boys versus girls who were TPMT heterozygous or homozygous deficient \((14 \text{ [14%] of 98 boys versus five [6.1%] of 82 girls]} \) \((P = .091)\).

**DISCUSSION**

6-Mercaptopurine is one of the most widely used medications for the treatment of childhood acute lymphoblastic leukemia. It has been previously shown that patients who inherit TPMT deficiency develop severe \((3,5–7)\) and potentially fatal \((8)\) hematopoietic toxicity when treated with conventional doses of 6-mercaptopurine \((i.e., 50–75 \text{ mg/m}^2 \text{ per day})\). However, TPMT-deficient patients can be safely treated with 6-mercaptopurine if they are given substantially lower doses \((i.e., 6\%–10\% \text{ of conventional doses})\) \((3,5,6)\). The mechanism of 6-mercaptopurine intolerance in TPMT-deficient patients is the absence of the principal metabolic inactivation pathway for thiopurines in hematopoietic tissue, TPMT-catalyzed \(S\)-methylation of 6-mercaptopurine and its thioguanine nucleotide metabolites \((19)\).

TPMT activity is inherited as an autosomal co-dominant trait: About one in 300 Caucasian, African, African-American, and Asian populations are TPMT deficient \((9–11,20,21)\), and approximately 10\% of these populations inherit intermediate TPMT activity due to heterozygosity at the TPMT locus. It was not previously known whether TPMT heterozygous individuals could tolerate 6-mercaptopurine doses comparable to homozygous wild-type patients or whether they were at a higher risk of dose-limiting 6-mercaptopurine toxicity. This study has established that this genetically defined subset of the population accumulates statistically significantly higher cellular levels of the active thioguanine nucleotide metabolites and experiences greater toxicity when treated with conventional doses of 6-mercaptopurine. This finding indicates that the genetic polymorphism of TPMT has a substantially greater influence on tolerance to acute lymphoblastic leukemia chemotherapy than would be the case if only the relatively rare TPMT-deficient patients were intolerant to full-dose 6-mercaptopurine.

This study has shown that, at conventional 6-mercaptopurine doses of 75 mg/m\(^2\) per day, TPMT heterozygotes accumulate approximately twofold more thioguanine nucleotides in their erythrocytes when compared with homozygous wild-type patients. This difference in thioguanine nucleotide accumulation translated into a fivefold greater cumulative incidence of 6-mercaptopurine dose-limiting toxicity in TPMT heterozygotes compared with TPMT wild-type patients \((Fig. 6; 35\% \text{ versus [7\% cumulative incidence]})\). Consistent with this finding, TPMT wild-type patients tolerated 75 mg/m\(^2\) per day of 6-mercaptopurine during 84\% of scheduled therapy compared with 65\% in heterozygous patients and only 7\% in TPMT-deficient patients \((Fig. 5)\). These data indicate that no TPMT-deficient patient will tolerate full-dose 6-mercaptopurine and that TPMT heterozygotes will require 6-mercaptopurine dose reductions significantly more often than TPMT-homozygous wild-type patients.

### Table 2. Rates* of primary toxic effects according to thiopurine \(S\)-methyltransferase phenotype

<table>
<thead>
<tr>
<th></th>
<th>Homozygous wild-type</th>
<th>Heterozygous</th>
<th>Homozygous mutant</th>
<th>(P^\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of weeks‡</td>
<td>1.4849</td>
<td>1.521</td>
<td>35</td>
<td>.035</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>0.50</td>
<td>0.07</td>
<td>0.00</td>
<td>.137</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1.28</td>
<td>2.63</td>
<td>20.00</td>
<td>.007</td>
</tr>
<tr>
<td>Weeks of missed MP due to neutropenia</td>
<td>8.24</td>
<td>14.88</td>
<td>25.7</td>
<td>.003</td>
</tr>
<tr>
<td>Weeks of missed MP</td>
<td>17.68</td>
<td>23.60</td>
<td>51.43</td>
<td>.128</td>
</tr>
<tr>
<td>Hospitalization (fever and neutropenia or infection)</td>
<td>4.67</td>
<td>3.56</td>
<td>11.40</td>
<td>.128</td>
</tr>
</tbody>
</table>

*Units = number of toxic events or missed therapy per 100 weeks at risk, up until the time that the 6-mercaptopurine (MP) dose was altered to prevent toxicity.

†Compares heterozygotes to homozygous wild-type by use of generalized estimation equations for longitudinal binary data.

‡Number of weeks in which MP was expected to be delivered up until the MP dose was altered to prevent toxicity. This excludes weeks when no MP was scheduled to be given.

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**Fig. 6.** Cumulative incidence (95\% confidence intervals) of requiring a decrease in 6-mercaptopurine dose (from 75 mg/m\(^2\) per day) to prevent toxicity among patients who were homozygous wild-type, heterozygous, and homozygous deficient \((P < .001)\). Values = final cumulative incidences at the end of therapy and are also indicated at 1 year for those with heterozygous or wild-type status.
While it is true that clinicians were encouraged to decrease the 6-mercaptopurine dose alone (rather than the methotrexate doses) in patients who had experienced toxicity, blood cell counts ultimately determined how much 6-mercaptopurine patients were able to tolerate. Thus, the polymorphic nature of the human TPMT gene identifies about 10% of patients who are at higher risk of acute 6-mercaptopurine toxicity because of an inherited deficiency in 6-mercaptopurine metabolism. It is interesting that the average dose eventually tolerated by TPMT heterozygotes was only 15% lower than the protocol dose tolerated by the patients with the wild-type TPMT phenotype, despite accumulating twice as high a concentration of thioguanine nucleotides in their red blood cells. The ability of patients with TPMT defects (homozygous deficient or heterozygous) to tolerate higher thioguanine nucleotide levels than those with wild-type TPMT (3,7,22) may be related to the substantially lower methylthioinosine monophosphate levels in these patients, since methylthioinosine monophosphate can be cytotoxic via inhibition of de novo purine synthesis (23). Consistent with this notion, very few patients with the wild-type TPMT phenotype could tolerate 6-mercaptopurine doses above 75 mg/m² per day (in our setting of relatively intense methotrexate dosing at 40 mg/m² per week parenterally), possibly because of high methylthioinosine monophosphate concentrations. Moreover, dose escalation can cause neutropenia, necessitating withholding of therapy until blood cell counts recover, which may compromise acute lymphoblastic leukemia outcome (24). In addition to being at higher risk of acute hematopoietic toxic effects, as described herein, patients with TPMT defects may also be at higher risk for irradiation-associated brain tumors (25) and etoposide-induced myeloid leukemia (26). Together, these data suggest that there is a subset of patients with TPMT defects who requires reductions in 6-mercaptopurine dose to avoid acute and long-term toxic effects, whereas increased doses in those wild-type for TPMT should be undertaken cautiously so as to avoid inducing excessive neutropenia.

Although there are data suggesting that intravenous 6-mercaptopurine is associated with acute hepatotoxicity (27), we found no evidence that hepatotoxicity was more frequent in those with TPMT defects. In fact, hepatotoxicity tended to be more frequent among those with higher TPMT activity (Table 2). Because hepatotoxicity followed administration of 6-methylmercaptopurine riboside (28–30), it is possible that methylated metabolites contribute to hepatotoxicity.

There are two strategies for prospectively identifying TPMT-deficient and heterozygous patients: either measure TPMT activity in erythrocytes (31,32) or determine TPMT genotype using genomic DNA (15,33). Measurement of erythrocyte TPMT activity is accomplished by either radiochemical (31) or HPLC (32) assays by use of a small volume of peripheral blood, but results can be spuriously high if patients have received allogeneic erythrocyte transfusions within the previous 60–90 days. Because it is not uncommon for newly diagnosed acute lymphoblastic leukemia patients or those experiencing hematopoietic toxicity to receive erythrocyte transfusions, this is frequently a serious limitation of diagnosing TPMT phenotype on the basis of TPMT activity. Alternatively, polymerase chain reaction-based methods have been developed to detect the signature mutations in the predominant TPMT mutant alleles in humans (10,15,20,21,34). Detection of the three most prevalent TPMT mutations yielded greater than 95% concordance between TPMT genotype and phenotype in a Caucasian population (15), and one can anticipate that the molecular diagnosis of TPMT deficiency and heterozygosity will continue to improve as additional mutations are discovered and incorporated into automated high throughput methods (e.g., DNA arrays).

Noncompliance, as assessed by undetectable erythrocyte thioguanine nucleotide concentrations, was rare (three of 180 patients) and comparable to rates reported in the U.K. (35). Only one of these three cases was suspected clinically, illustrating the utility of pharmacologic measures in documenting noncompliance.

Defining cut points to divide patients into phenotypes according to their TPMT activity is challenging because of the increase in activity of TPMT that occurs while patients are receiving chemotherapy (4,36,37). We acknowledge that our sample size was too small to apply statistical methods to accurately identify an antimode dividing heterozygotes from wild-type for TPMT, as we have done previously in healthy volunteers (11). By the use of cut points previously validated in large family studies (9) and against genotype (15) for those who had already completed therapy and by the use of cut points that identified the intermediate 10% of the group as presumed heterozygotes for those measures taken while patients were receiving therapy, phenotype was consistent with genotype and our cut points are similar to those reported by others (34).

Whether boys differ from girls in amount of 6-mercaptopurine prescribed, TPMT activity, or thioguanine nucleotide concentrations has differed in different studies (38–40), with lower dosing in boys postulated to contribute to their worse acute lymphoblastic leukemia outcome. However, we did not observe any differences in thioguanine nucleotides, TPMT (41), or in 6-mercaptopurine dosing between boys and girls.

It is important to recognize that, when full doses of 6-mercaptopurine are prescribed to patients with undiagnosed TPMT deficiency or heterozygosity, this can compromise the ability to deliver all forms of acute lymphoblastic leukemia chemotherapy and thereby jeopardize the chance for cure. However, with the appropriate dose adjustment of 6-mercaptopurine (or thioguanine or azathioprine), TPMT-deficient and heterozygous patients can be successfully treated with all components of acute lymphoblastic leukemia therapy including thiopurines (3). This study establishes that this inherited trait is a significant determinant of tolerance to acute lymphoblastic leukemia chemotherapy that contains 6-mercaptopurine (or other thiopurines). Because this common genetic polymorphism places approximately 10% of the patients at risk for excessive toxicity, we suggest that TPMT phenotype should be established in patients to optimize their thiopurine therapy.

REFERENCES

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NOTES

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