Thiopurine methyltransferase (TPMT) is not a major risk factor for secondary malignant neoplasms after treatment of childhood acute lymphoblastic leukemia on Berlin-Frankfurt-Münster protocols

*Martin Stanulla,* Elke Schaeffeler, Anja Mörcke, Sally A. Coulthard, Gunnar Cario, André Schrauder, Peter Kaatsch, Michael Dördelmann, Karl Welte, Martin Zimmermann, Alfred Reiter, Michel Eichelbaum, Hansjörg Riehm, Martin Schrappe, and Matthias Schwab

1University Children's Hospital, Kiel, Germany; 2Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany; 3Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom; 4German Childhood Cancer Registry, Institute of Medical Biometrics, Epidemiology, and Informatics (IMBEI), Mainz, Germany; 5Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany; 6Pediatric Hematology and Oncology, University Children's Hospital Giessen, Giessen, Germany; and 7Department of Clinical Pharmacology, University Hospital Tübingen, Tübingen, Germany

Introduction

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that is ubiquitously expressed in the human body and catalyzes the S-methylation of thiopurine drugs, such as azathioprine, 6-mercaptopurine, and 6-thioguanine. The TPMT locus underlies a genetic polymorphism with heterozygotes having intermediate and homozygous variant people having low TPMT activity. To date, at least 24 mutant alleles responsible for variation in TPMT enzyme activity have been described. The most frequent of these alleles (TPMT*2 and *3) explain more than 95% of defective TPMT activity. TPMT genotype is highly concordant with TPMT phenotype.

The 6-mercaptopurine and to a lesser extent 6-thioguanine are regularly administered during the treatment of acute lymphoblastic leukemia (ALL). Of importance with regard to potential long-term adverse effects related to thiopurine treatment, patients with childhood ALL and diminished TPMT activity were shown to be at increased risk of developing therapy-associated acute myeloid leukemia and brain tumors. In the present study, we genotyped 105 of 129 patients who developed a secondary malignant neoplasm after ALL treatment on 7 consecutive German Berlin-Frankfurt-Münster trials for all functionally relevant TPMT variants. Frequencies of TPMT variants were similarly distributed in secondary malignant neoplasm patients and the overall ALL patient population of 814 patients. Thus, TPMT does not play a major role in the etiology of secondary malignant neoplasm after treatment for childhood ALL, according to Berlin-Frankfurt-Münster strategies. (Blood. 2009;114:1314-1318)

Methods

Patients

Through systematically searching our database, we identified 129 of 9139 ALL patients who were treated in Germany in 1 of 7 consecutive ALL-BFM multicenter trials since 1979 and subsequently developed a SMN (Table 1). The median follow-up for the entire patient cohort was 8.1 years as of June 30, 2006; 40% of the entire ALL-BFM patient cohort had a documented follow-up of more than 10 years. Treatment is described elsewhere and contained comparable multidrug chemotherapeutic regimens and, in parts of the patient population, cranial irradiation (CI) and/or hematopoietic stem cell transplantation. With the exception of ALL-BFM 79 (2 branches), treatment was stratified into 3 branches (standard, intermediate, and high risk), mainly according to the initial leukemic cell load, adverse genetic aberrations such as t(9;22) and t(4;11), and treatment response. Briefly, all ALL-BFM trials included in the present analysis made and keep extensive use of thiopurines, with 6-mercaptopurine (60 mg/m² per day) first being applied for 4 weeks during consolidation in the second phase of BFM protocol I. Comedication during this period consists of cytarabine, cyclophosphamide, and intrathecal methotrexate. In the subsequent central nervous system (CNS)–directed treatment protocol, introduced through a


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1314 BLOOD, 13 AUGUST 2009 • VOLUME 114, NUMBER 7
randomized study question in ALL-BFM 81, 6-mercaptopurine (25 mg/m² per day) was given concomitantly with intravenous methotrexate (0.5 g/m² x 4 x 24 h in ALL-BFM 81 and 83; 5 g/m² in the following trials). During reintensification, 6-thioguanine (60 mg/m² per day) is applied for 2 weeks in the second phase of BFM protocol II. Comedication during this period consists of cytarabine, cyclophosphamide, and intrathecal methotrexate.

Maintenance treatment, including interim maintenance in trials ALL-BFM 79 and 81, contained 50 mg/m² per day 6-mercaptopurine and methotrexate 20 mg/m² per week. Before intravenous methotrexate had been introduced, CI was applied during the second half of BFM protocol I (between 5 and 12 weeks from diagnosis) and since study ALL-BFM 83, CI has been given at the end of reintensification (after approximately 7 months from diagnosis). At both of these time points, there was and is concomitant application of 6-mercaptopurine (50 mg/m² per day). ALL-BFM 79 and 81 were the only studies with intrathecal methotrexate being applied concomitantly with CI. Doses for CI have been reduced since study ALL-BFM 79, in which standard-risk patients received 18 Gy (<2 years, 15 Gy; <1 year, 12 Gy) and high-risk patients 24 Gy (<2 years, 19 Gy; <1 year, 16 Gy). CI was first eliminated for low standard-risk patients of study ALL-BFM 83. Mainly depending on the tumor load, ALL-BFM 86 used only 12 Gy for all nonstandard-risk patients. Standard-risk patients did not receive CI. Since ALL-BFM 86, CNS-positive patients (mainly defined through >105 leukocytes/μL cerebrospinal fluid with definable blasts) received a dose of 24 Gy (<2 years, 18 Gy; <1 year, no CI). In the subsequent trials, ALL-BFM 95 and ALL-BFM 2000, preventive CI at 12 Gy was only applied in T-cell ALL and high-risk patients; CNS-positive patients received 18 Gy (<2 years, 12 Gy; <1 year, no CI).

Of 129 SMN patients identified, 105 patients, representing 81.4% of the entire SMN patient population, had archival peripheral blood or bone marrow smears or previously isolated DNA available and could be genotyped for TPMT. A slight overrepresentation of hematologic SMN and a similar underrepresentation of brain tumors were observed for the
Table 2. *TPMT* genotype in 814 patients with childhood ALL consecutively enrolled in trial ALL-BFM 2000 (reference cohort) and in 105 patients developing a SMN after treatment on ALL-BFM trials 79 to 2000

<table>
<thead>
<tr>
<th><em>TPMT</em> genotype</th>
<th>Reference cohort (n = 814)</th>
<th>All SMN patients (n = 105)</th>
<th>Hematologic SMN (n = 60)</th>
<th>Hematologic SMN, AML/MDS only (n = 41)</th>
<th>Brain tumor SMN (n = 21)</th>
<th>Solid tumor SMN (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>Odds ratio (95% CI)</td>
<td>n (%)</td>
<td>Odds ratio (95% CI)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Wild-type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>755 (92.8)</td>
<td>98 (93.3)</td>
<td>1.00</td>
<td>56 (90.2)</td>
<td>1.00</td>
<td>20 (95.2)</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*2</td>
<td>3 (0.4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>*1/*3A</td>
<td>42 (5.2)</td>
<td>6 (5.7)</td>
<td>3 (5.0)</td>
<td>3 (7.3)</td>
<td>1 (4.8)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>*1/*3C</td>
<td>9 (1.1)</td>
<td>1 (1.0)</td>
<td>1 (1.7)</td>
<td>1 (2.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>*1/*9</td>
<td>1 (0.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total heterozygotes*</td>
<td>55 (6.8)</td>
<td>7 (6.7)</td>
<td>0.98 (0.43-2.21), P = .93</td>
<td>4 (6.7)</td>
<td>0.98 (0.34-2.80), P = .97</td>
<td>4 (9.8)† 1.48 (0.51-4.31), P = .47</td>
</tr>
<tr>
<td>Deficients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*2/*3A</td>
<td>1 (0.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>*3A/<em>3A</em></td>
<td>2 (0.2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>*3A/<em>11B</em></td>
<td>1 (0.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total deficients</td>
<td>4 (0.4)</td>
<td>n.c.</td>
<td>n.c.</td>
<td>n.c.</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; MDS, myelodysplastic syndrome; n.c., not calculated; and —, not observed.

*Multivariate odds ratios and 95% CI for *TPMT* heterozygosity, including immunophenotype, the only variable demonstrating a tendency of being associated with both development of a SMN (P = .09) as well as *TPMT* genotype (P = .07) in the model: all patients, odds ratio 0.93, 95% CI 0.41-2.11; hematologic SMN, odds ratio 0.91, 95% CI 0.31-2.82; AML/MDS only, odds ratio 1.42, 95% CI 0.49-4.15; brain tumor SMN, odds ratio 0.59, 95% CI 0.07-4.59; other solid tumor SMN, odds ratio 1.28, 95% CI 0.29-5.65.

†Two of the 4 patients presented with AML and 2 with MDS.
included 105 SMN patients compared with those 24 patients not included (Table 1). Only 2 of the 129 SMN patients underwent hematopoietic stem cell transplantation for treatment of their primary ALL.

Follow-up data for patients were maintained through regular submissions of reports from the respective treatment centers in Germany. For the first 5 to 10 years of follow-up, reports were filed on an annual basis. After this period, up to adulthood reports were filed on a biannual basis. For adolescents and adults no longer returning to their pediatric treatment centers, but who consented for being further contacted, the nationwide operating German Childhood Cancer Registry in Mainz conducts an extended follow-up based on 3- to 5-year intervals. In case of SMN identification through the latter procedure, the principal trials are informed and help to secure the validity of the information.

The ALL-BFM patient cohort used as a reference population for comparing TPMT genotype distributions consisted of 814 patients representative of the total patient population (n = 956) enrolled in trial ALL-BFM 2000 from October 1999 to September 2002.15 This study was approved by the institutional review board of the Hannover Medical School.

TPMT genotyping

DNA extraction from smears or fresh mononuclear cells was performed, as described previously.16 In addition to standard genotyping for the variant TPMT*2 and TPMT*3 alleles,17 we performed a comprehensive screen for all currently known TPMT variant alleles conferring diminished enzyme activity (TPMT*2 to *18 and *20 to *23) by using a matrix-assisted laser desorption ionization–time of flight mass spectrometry method.3 Laboratory staff was blinded to the case status of study participants.

Statistical analysis

Differences in the distribution of categorical variables were analyzed by χ² or Fisher exact tests.Observed and expected allele and genotype frequencies within populations were compared by Hardy-Weinberg equilibrium calculations (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The association of TPMT variants with risk of SMN was examined by use of unconditional logistic regression analysis to calculate odds ratios and their 95% confidence intervals. P values less than .05 were considered statistically significant. The SPSS statistical package (SPSS) was used for computerized calculations.

Results and discussion

Our analyses did not reveal a higher frequency of TPMT genotypes associated with decreased TPMT enzyme activity among SMN patients compared with a reference cohort of 814 childhood ALL patients (Table 2). Similarly, in multivariate analyses including immunophenotype (categories: precursor B and T cell), the only variable demonstrating a tendency of being associated with both development of a SMN (P = .09) as well as TPMT genotype (P = .07), and stratified analyses by different entities of SMN, no significant associations with genotypes conferring lower TPMT activity have been observed (Table 2). Additional comprehensive genotyping results obtained by using matrix-assisted laser desorption ionization–time of flight mass spectrometry,3 including all currently known clinically relevant TPMT variants, were in perfect agreement with those results generated by standard genotyping.17 No further TPMT variants were detected.

Previous reports in the literature described a relationship of heterozygous or homozygous variant TPMT phenotypes with SMN after treatment for childhood ALL. In a study conducted at St Jude Children’s Research Hospital (SJCRH), Total Therapy Study XIIHR, patients with lower TPMT activity showed a trend toward a higher incidence of tAML (P = .16) associated with the application of the topoisomerase II inhibitor etoposide.9 In the Scandinavian Nordic Society of Pediatric Haematology and Oncology (NOPHO) ALL-92 trial, Thomsen et al reported on a significantly higher risk of tAML or myelodysplastic syndrome in patients with lower TPMT activity compared with control patients (P = .03), resulting in 6-thioguanine nucleotide levels in red blood cells higher than the 92nd percentile of all patients.10 The main and probably most important difference between the SJCRH and NOPHO protocols in comparison with BFM protocols for treatment of childhood ALL is that 6-mercaptopurine starting doses for initiation of maintenance treatment are lower on BFM protocols (50 vs 75 mg/m² per day). A second difference relates to the 6-mercaptopurine dose given concurrently with high-dose methotrexate. Whereas on SJCRH and NOPHO protocols 75 mg/m² per day 6-mercaptopurine is administered with high-dose methotrexate, patients on BFM protocols only received 25 mg/m² per day. This may be important, as 6-mercaptopurine and methotrexate act synergistically through the inhibition of purine de novo synthesis, leading to a higher intracellular availability and increased incorporation of phosphorylated thiopurines in DNA and RNA.19,20 Moreover, animal models indicate that antimetabolites contribute to tumorigenesis.21 Of interest in this context, TPMT heterozygotes were suggested to be at increased risk of developing myelotoxicity when high-dose methotrexate is administered with concurrent oral 6-mercaptopurine at a dose of 75 mg/m² per day.22,23

In a second study conducted at SJCRH, Total Therapy Study XII, Relling et al reported on a higher incidence of brain tumors in childhood ALL patients with lower TPMT activity who had received CI concurrent with 6-mercaptopurine in the initial maintenance phase.31 Six of 52 patients receiving CI and concurrent 6-mercaptopurine developed a brain tumor. Of these 6 patients, 4 had red blood cell 6-thioguanine nucleotide levels above the 70th percentile for the entire cohort of 52 patients, and 3 have had intermediate or very low TPMT activity. The 8-year cumulative incidence of brain tumors among children with low TPMT activity was 42.9% plus or minus 20.6% versus 8.3% plus or minus 4.7% in TPMT wild-type patients. Whereas on the NOPHO ALL-92 protocol patients did not regularly receive CI,26 6-mercaptopurine application concurrent with CI during early maintenance was probably lower on BFM compared with SICRH protocols, as recommended starting doses on the respective protocols differed (50 vs 75 mg/m² per day).8,25 Other differences that only apply to the comparison of BFM and SJCRH protocols relate to topoisomerase II inhibitors, which on BFM protocols are not given in close association with thiopurines, and to intrathecal triple therapy (methotrexate, cytarabine, and a glucocorticoid), which on BFM protocols is not given concurrently with CI and 6-mercaptopurine.8,25

Finally, we cannot exclude selection bias as an additional explanation for the results presented in this study. Only 40% of the entire cohort of ALL-BFM patients had a documented follow-up of more than 10 years. Thus, an incomplete ascertainment of SMN cases may have influenced our analyses.

In conclusion, low activity TPMT does not confer an increased risk of SMN after therapy for childhood ALL when treated according to BFM strategies. This is most likely explained by differences between clinical protocols regarding the intensity of thiopurine treatment and/or application in the context of other chemotherapeutic and/or radiotherapeutic exposures.

Acknowledgments

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Authorship

 Contribution: M. Stanulla and M. Schwab designed the study, analyzed the data, and wrote the manuscript; E.S. and S.A.C. performed research and contributed to writing of the manuscript; G.C., A.S., P.K., M.D., A.R., K.W., and H.R. collected data and contributed to writing of the manuscript; A.M. and M.Z. analyzed data and contributed to writing of the manuscript; and M.E. and M. Schrappe were involved in the initiation of the study, took part in designing the study, and contributed to writing of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Dr Martin Stanulla, Department of Pediatrics, University Children’s Hospital, Arnold Heller Str 3, Haus 9, 24105 Kiel, Germany; e-mail: martin.stanulla@uk-sh.de.

References

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