Pharmacogenetically Based Dosing of Thiopurines in Childhood Acute Lymphoblastic Leukemia: Influence on Cure Rates and Risk of Second Cancer

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Background. Previous studies have indicated that patients with thiopurine methyltransferase (TPMT) low activity (TPMTLA) have reduced risk of relapse but increased risk of second malignant neoplasm (SMN) compared to patients with TPMT wild-type (TPMTWT) when treated with 6MP maintenance therapy starting doses of 75 mg/m²/day. To reduce SMN risk, 6MP starting doses were reduced to 50 mg/m²/day for patients with TPMT heterozygosity in the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2000 protocol. Procedure. We explored the pattern of SMN and relapse in the NOPHO ALL2000 protocol (n = 674) and NOPHO ALL92 protocol (n = 601) in relation to TPMT phenotypic- and/or genotypic. Results. The overall risk of any event did not differ significantly between the two protocols. However, in event pattern analyses considering only the patients with TPMTLA who experienced relapse or SMN, the risk of SMN versus leukemia relapse was significantly lower in the ALL2000 cohort for patients with 6MP starting dose <75 mg/m²/day when compared to the patients in ALL92 (relapse (n = 11) and SMN (n = 0)) in ALL2000 versus relapse (n = 5) and SMN (n = 4) in ALL92, P = 0.03). Furthermore, the 8-year cumulative incidence of relapse for patients with TPMTLA was significantly higher in the ALL2000 compared to the ALL92 cohort (19.7% (11.6–33.3%) vs. 6.7% (2.9–15.5%), P = 0.03). Conclusion. This study indicates that reducing 6MP starting dose for patients with TPMTLA may reduce SMN risk but lead to a relapse risk similar to that of patients with TPMTWT. Pediatr Blood Cancer 2014;61:797–802. © 2014 Wiley Periodicals, Inc.

INTRODUCTION

Dosing of thiopurines according to thiopurine methyltransferase (TPMT) genotype is the only example of routine pharmacogenetically based drug dosing in childhood acute lymphoblastic leukemia (ALL) protocols [1–4], but the benefits of this strategy are uncertain. The widely used thiopurine, 6-mercaptopurine (6MP), primarily exerts its cytotoxicity through conversion into 6-thioguanine nucleotides (6TGN) that are incorporated into DNA and cause DNA-damage [5–7]. TPMT methylates thiopurines, including several metabolites, and this competes with the formation of 6TGN. Approximately 10% of Caucasians are heterozygous for the TPMT low activity allele, and one in 300 individuals has TPMT deficiency with two low activity alleles [8].

In the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL92 protocol, all patients received a maintenance therapy 6MP starting dose of 75 mg/m²/day. In that study, patients with low TPMT activity (TPMTLA) had a significantly reduced risk of relapse compared to patients with TPMT wild-type (TPMTWT) (7% vs. 18%, P = 0.03) [9], but also an increased risk of developing a second malignant neoplasm (SMN) (6% vs. 2%, P = 0.06) [10,11]. In contrast, a Berlin-Frankfurt-Münster (BFM) study showed no increased risk of SMN among the patients with TPMTLA [12]. Since, (i) this discrepancy could reflect lower starting doses of 6MP (50 mg/m²/day) in the BFM protocol, (ii) the median 6MP dose actually prescribed for patients with TPMT heterozygosity on NOPHO ALL92 during maintenance therapy was approximately 50 mg/m²/day [9,11], and (iii) the degree of myelotoxicity was not related to relapse rates for patients with TPMT heterozygosity in the NOPHO ALL92 study [9], the recommended starting dose of 6MP was reduced from 75 to 50 mg/m²/day for patients with TPMT heterozygosity in the NOPHO ALL2000 protocol. In the present study of 674 patients

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in NOPHO ALL2000 with available data on TPMT genotype, we explored the impact of this strategy on risks of relapse and SMN.

PATIENTS AND METHODS

Patients

Patients were eligible if they were (i) non-Down; (ii) diagnosed with B-cell precursor (BCP) or T-cell ALL in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) between January 2002 and June 2008; (iii) t(9;22)(q34;q11)|BCR-ABL| negative; (iv) between 1.0 and 14.9 years of age when diagnosed; (v) treated according to the NOPHO ALL2000 protocol; (vi) non-transplanted; (vii) in first remission at start of maintenance therapy; and (viii) with available TPMT genotype. These eight criteria were fulfilled by 674 patients.

Risk Group Assignment

Risk group assignment was based on age, white blood cell count (WBC) at diagnosis and presence of higher-risk features. Standard risk (SR): age 1.0–9.9 years, WBC <10 × 10^9/L and no higher-risk features; intermediate risk (IR): age ≥10 years and/or WBC 10–49.9 × 10^9/L and no higher-risk features; and higher-risk (HR): WBC ≥50 × 10^9/L and/or presence of higher-risk features: T-lineage ALL, CNS or testicular involvement, translocations t(9;22)(q34;q11)|BCR-ABL|, t(4;11)(q21;q23)|AF4/MLL| or t(1;19)(q23;p13)|E2A-PBX1|, hypodiploidy (<45 chromosomes), MLL-rearrangements, lymphomatous leukemia, mediastinal lymphoma, and poor treatment response (M3 bone marrow (BM) at day 15 or M2/M3 BM at day 29). Patients with higher-risk features were assigned to the very high risk (VHR) treatment arm, if they had WBC of 100–199 × 10^9/L and/or T-cell disease with mediastinal mass and/or CNS leukemia; since patients on the VHR treatment arm were given cranial irradiation, they needed to be greater than 5 years of age. Patients were stratified to the extra high risk (EHR) group (and cranial irradiation, they needed to be greater than 5 years of age).

Cytogenetics

G-band karyotyping as well as fluorescent in situ hybridization and/or reverse transcriptase PCR for translocations t(9;22)(q34; q11)|BCR-ABL|, t(1;19)(q23;p13)|E2A-PBX1|, and 11q23/MLL-rearrangements were mandatory in the NOPHO ALL2000 protocol. Nearly all patients were also examined for the translocation t(12;21)|ETV6-RUNX1|, and many were explored with comparative genomic hybridization, spectral karyotyping and/or DNA-index by flow cytometry. All cytogenetic results were subsequently scrutinized centrally by the NOPHO cytogenetic working group and scored according to ISCN 1995 [13]. To investigate the influence of TPMT genotype on risks of relapse and SMN, patients were divided into four cytogenetic subsets: (i) those with favorable karyotypes (high hyperdiploidy with modal chromosome numbers of 51–61 or a t(12;21)|ETV6-RUNX1| translocation), (ii) those with unfavorable karyotypes (hypodiploidy with modal chromosome numbers <45, t(1;19)(q23;p13)|E2A-PBX1| translocation, or 11q23/MLL-aberrations), (iii) those with other cytogenetic aberrations, and (iv) those lacking karyotyping or with no aberrant karyotype.

Treatment

Based on risk group assignment, patients were treated according to the SR, IR, HR, VHR or EHR arm [14]. Apart from 16 cases, all patients received treatment according to the risk group criteria. In the following analyses, patients were grouped by the treatment they actually received. All patients initiated treatment with induction and consolidation therapy. Patients treated according to the IR, HR or VHR arm received delayed intensification, and patients assigned to the VHR treatment arm received 18 Gy cranial irradiation before entering maintenance therapy (Supplemental Table I) [14,15].

6MP/MTX maintenance therapy was initiated at treatment weeks 17 (SR), 30 (IR), 70 (HR), or 61 (VHR) and continued until 2.0 (HR and VHR) or 2.5 years (SR and IR) from diagnosis. The starting dose of oral MTX was 20 mg/m^2/week, and the starting dose of oral 6MP was adjusted according to the TPMT genotype (TPMT^WT: 75 mg/m^2/day; TPMT heterozygous: 50 mg/m^2/day; TPMT deficient: 5–10 mg/m^2/day). Subsequently, MTX and 6MP doses were adjusted to a target WBC of 1.5–3.5 × 10^9/L. During the first year of MTX/6MP maintenance therapy, patients with SR- or IR-ALL received alternate pulses at 4 weeks intervals of (i) vincristine (2 mg/m^2 once, maximum 2.5 mg) and dexamethasone (6 mg/m^2/day for 5 days) and (ii) HD-MTX (5 g/m^2/24 hours) with intrathecal MTX and leucovorin rescue until five courses of HD-MTX had been given. Every 4 weeks throughout 6MP/MTX maintenance therapy, patients assigned to the HR or VHR treatment arm received reinductions of vincristine (2.0 mg/m^2 once, maximum 2.5 mg) and dexamethasone (6 mg/m^2/day for 5 days).

Thiopurine Methyltransferase

TPMT genotype was evaluated for presence of low activity variants c.460G>A and/or c.719A>G. Of the 674 included patients, 617 were classified as TPMT^WT, 56 (8.3%) as TPMT heterozygous and one (0.1%) as TPMT deficient. Patients with TPMT heterozygosity or TPMT deficiency were grouped together as patients with TPMT low activity (TPMT^LA). DNA for confirmatory TPMT genotype analysis could as part of the present study be collected for 12 of the 18 patients who developed SMN or patients with TPMT^LA experiencing an event. The genotype was confirmed in all cases.

Physician Compliance to the Protocol Guidelines

To analyze the physician compliance to the dose adjustment guidelines for patients with TPMT^LA in ALL2000, the maintenance therapy starting dose of 6MP was collected for patients registered as TPMT^LA at study initiation. Furthermore, we determined drug doses and blood counts during maintenance therapy, but due to the complexity in collecting these data, drug doses were only obtained for 12 of the 13 patients with TPMT^LA who developed relapse (n = 11) or SMN (n = 1) and blood counts were only obtained for 9 of these 13 patients with TPMT^LA and event (relapse (n = 8) or SMN (n = 1)). Characteristics on the 13 patients with TPMT^LA who developed an event are shown in Table I. The data of these patients were compared to the patients with TPMT^LA in the NOPHO ALL92 study cohort being in first remission at the start of maintenance therapy. Of the 1,645 patients treated by the NOPHO ALL92
protocol, TPMT pheno- and/or genotype was available for 609 patients (TPMTLA, n = 76). Of the 609 patients, four developed a relapse or died in first remission before initiating maintenance therapy and were not included in the study. Furthermore, four patients treated with LSA1/L2 maintenance therapy were not included leading to a total of 601 patients (TPMT LA, n = 75) [9]. Of the 75 patients with TPMTLA, 61 participated in the randomized ALL92 study of individualized dose adjustments of 6MP and MTX during maintenance therapy with a total of 28,580 data sets on the NOPHO ALL92 cohort. For each patient, the dose history was compared to data of similar patients with TPMTLA in the NOPHO ALL2000 cohort, the prescribed doses of MTX and 6MP were derived by the standard error of the difference and assuming an asymptotic normal distribution. Approximate point-wise 95% confidence intervals were based on the log-transformation.

Multivariable Cox regression analysis [19] was performed to assess the relative risk of relapse for patients with TPMTLA compared to patients with TPMTWT. In this analysis, the patients who experienced a competing event were censored at the time point of these events. The multivariable model was adjusted for sex, risk group, age at diagnosis, WBC at diagnosis, BCP/T-cell ALL, karyotype, and TPMT genotype. The proportional hazards assumption was assessed by the score processes using graphical methods [20] and the Lin, Wei, and Ying test [21]. Risk group was used as a stratification variable, whereas the other variables met the assumption of proportional hazards. The Wald test was applied to test for differences in outcome.

For the patients with TPMTLA experiencing an event in the NOPHO ALL2000 cohort, the prescribed doses of MTX and 6MP were compared to data of similar patients with TPMTLA in the NOPHO ALL92 cohort. For each patient, the dose history was established on a daily basis by letting each registration of the doses count until a new registration (maximum, 8 weeks). To compare the doses for the two groups of patients visually, locally weighted polynomial regression (lowess regression) [22] was performed for the patients with TPMTLA subsequently developed an event, of whom data on drug doses and blood counts during maintenance therapy was available for eight out of nine patients (relapse (4 of 5), SMN (1 of 5)).

**Statistics**

In the statistical analyses patients treated according to the HR, VHR or EHR treatment arm were merged to one group named HR-ALL. The differences between categorical variables were examined using Pearson chi-square or Fisher’s exact tests. The differences between continuous variables were examined by the Mann–Whitney U or Kruskal–Wallis tests. The average WBC count, absolute neutrophil count (ANC) and thrombocyte count during maintenance therapy were calculated as weighted means using as weight the interval between the sample in question and the next [17]. Estimation of cumulative incidences were based on the Aalen–Johansen estimator [18] with delayed entry at the time point of initiation of maintenance therapy. Relapse, SMN and DCR1 were considered as competing events. The basic time scale was defined by the date of diagnosis. Tests for comparison of cumulative incidences were performed by comparing the estimated cumulative incidences at 8 years of follow-up, that is, constructing a landmark test statistic by the difference in estimated cumulative incidence divided by the standard error of the difference and assuming an asymptotic normal distribution. Approximate point-wise 95% confidence intervals were based on the log-transformation.

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committees in all Nordic countries. Informed consent was obtained according to the Declaration of Helsinki.

RESULTS

Of the 674 included patients in ALL2000, the median follow-up time for the 561 patients who remained in first remission was 7.3 years (50% range, 6.0–8.7 years). Three patients died in first remission, (all TPMT\textsuperscript{WT}), 104 patients developed a relapse of ALL 1.0 to 10.4 years from diagnosis (median, 2.9 years) (92 TPMT\textsuperscript{WT}, 12 TPMT\textsuperscript{LA}), and six patients developed an SMN 2.5–5.2 years from diagnosis (median, 3.3 years) (5 TPMT\textsuperscript{WT}, 1 TPMT\textsuperscript{LA}) (Table II). The overall 8-year event-free survival (pEFS\textsubscript{8y}) was 82.5% (79.6–85.5%). When comparing the patients with TPMT\textsuperscript{WT} and TPMT\textsuperscript{LA}, they did not differ significantly with respect to event-free survival (pEFS\textsubscript{8y}, 82.9% (79.8–86.0%) vs. 78.6% (68.5–90.1%); \(P = 0.22\)).

Overall, the risk of any event did not differ between the two protocols. However, in event pattern analyses considering only the patients with TPMT\textsuperscript{LA} who experienced relapse or SMN, the risk of SMN was lower in the ALL2000 cohort for patients with 6MP starting dose <75 mg/m\textsuperscript{2}/day when compared to patients on ALL92 (ALL2000: relapse (n = 11) and SMN (n = 0) vs. ALL92: relapse (n = 5) and SMN (n = 4), \(P = 0.03\)). The only patient with TPMT\textsuperscript{LA} in the ALL2000 cohort who developed SMN received 75.8 mg 6MP/m\textsuperscript{2}/day as starting dose.

The 8-year cumulative relapse risk for patients with TPMT\textsuperscript{LA} was significantly higher in the ALL2000 cohort compared to the ALL92 cohort (19.7% (11.6–33.3%) vs. 6.7% (2.9–15.5%); \(P = 0.03\)). There was no significant difference in risk of relapse when comparing patients with TPMT\textsuperscript{WT} in the ALL2000 and ALL92 cohort (15.8% (13.0–19.3%) vs. 16.1% (13.4–19.4%)). The 8-year cumulative SMN risk for patients with TPMT\textsuperscript{LA} was non-significantly lower in the ALL2000 than in the ALL92 cohort (1.8% (0.3–12.0%) vs. 4.0% (1.3–12.0%); \(P = 0.43\)) (Fig. 1).

The overall 8-year cumulative SMN risk among all 674 patients in the ALL2000 cohort was 0.9% (0.4–2.0%). Similar to the findings in the ALL92 cohort [11] patients on ALL2000 with SR-ALL had the greatest 8-year cumulative risk of SMN (1.9% (0.8–4.7%)) compared to patients with IR-ALL or HR-ALL (0.4% (0.1–3.0%) and 0%, respectively) (Fig. 2). With exception of one case, all SMNs occurred after cessation of maintenance therapy. Three of the six SMNs were therapy-related myeloid neoplasm, all diagnosed within 5.2 years from diagnosis. The remaining three patients developed lymphomas or lymphoproliferative disease. The risk of

![Fig. 1.](image1)

**Fig. 1.** Cumulative risks of relapse or second malignant neoplasm (SMN) for patients with thiopurine methyltransferase low activity in the NOPHO ALL92 and NOPHO ALL2000 studies. pRelapse\textsubscript{8y}: NOPHO ALL2000 (n = 57), 19.7% (11.6–33.3%); NOPHO ALL92 (n = 75), 6.7% (2.9–15.5%); \(P = 0.03\). pSMN\textsubscript{8y}: NOPHO ALL2000, 1.8% (0.3–12.0%); NOPHO ALL92, 4.0% (1.3–12.0%); \(P = 0.43\).

SMN did not differ significantly between the patients with TPMT\textsuperscript{WT} (5/617; pSMN\textsubscript{8y} = 0.8% (0.4–2.0%)) and the patients with TPMT\textsuperscript{LA} (1/57; pSMN\textsubscript{8y} = 1.8% (0.3–12.0%)) (\(P = 0.44\)) (Fig. 3).

The 8-year cumulative relapse risk did not differ significantly between the patients with TPMT\textsuperscript{WT} (92/617; 15.8% (13.1–19.1%)) and the patients with TPMT\textsuperscript{LA} (12/57; 19.7% (11.6–33.3%)) in the ALL2000 cohort (\(P = 0.48\)) (Fig. 3), and these two groups, TPMT\textsuperscript{WT} and TPMT\textsuperscript{LA}, were comparable with respect to sex, age, WBC groups, immunology, karyotype subsets, and risk group (\(P > 0.10\) for all comparisons) (Supplemental Table II). In a multivariable Cox regression analysis adjusted for sex, age at diagnosis, WBC at diagnosis, BCP/T-cell ALL, karyotype, and risk

![Fig. 2.](image2)

**Fig. 2.** Cumulative risk of a second malignant neoplasm (SMN) with respect to risk groups. pSMN\textsubscript{8y} 1.9% (0.8–4.7%) for patients with standard risk ALL (n = 261), 0.4% (0.1–3.0%) for patients with intermediate risk ALL (n = 237), and 0% for patients with higher-risk ALL (n = 176).

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<th>Table II. Event Distribution in the NOPHO ALL92 and ALL2000 Studies According to Thiopurine Methyltransferase Status</th>
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TPMT, indicates thiopurine methyltransferase; DCR, death in first remission; and SMN, second malignant neoplasm.

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patients with TPMT 1.8 vs. 1.6/C2 dose of 6MP (median, 53.6 vs. 66.3 mg/m²/day; < had their maintenance therapy starting dose of 6MP reduced to TPMT Pediatr Blood Cancer

DISCUSSION

was 18.9% (10.9–32.3%).

Fig. 3. Cumulative risks of a second malignant neoplasm (SMN) or relapse with respect to thiopurine methyltransferase genotype. SMN: Patients with TPMT wild-type (n = 617); pSMN8y 0.8% (0.4–2.0%) and patients with TPMT low activity (TPMT heterozygous (n = 56) or TPMT deficient (n = 1)); pSMN8y 1.8% (0.3–12.0%); Overall P-value for difference between TPMT groups: 0.44. Relapse: Patients with TPMT wild-type; pRELAPSE8y 15.8% (13.1–19.1%) and patients with TPMT low activity; pRELAPSE8y 19.7% (11.6–33.3%); Overall P-value for difference between TPMT groups: 0.48. TPMT indicates thiopurine methyltransferase.

Although the risk of SMN is only a few percent in most protocols, some subgroups of patients may have significantly higher-risk of SMN [25]. Thus, for patients with SR-ALL one-third of all deaths are caused by toxic deaths. Increased SMN incidence may reflect both genetic predisposition for certain SMN among the subset of leukemias allocated to the SR group (e.g., high hyperdiploidy [11] and ETv6-RUNX1-translocated ALL) and a relatively longer exposure to 6MP/MTX maintenance therapy [11,25].

A few studies from NOPHO and St Jude Children’s Research Hospital (SJCRH) have shown associations between TPMT genotype and/or phenotype and relapse rates and/or SMN risk [9,11,26,27]. Importantly, in both the NOPHO and SJCRH protocols the starting dose of 6MP is 75 mg/m²/day. In contrast, a BFM-study showed no association between TPMT genotype and risk of SMN when treating all patients with 50 mg 6MP/m²/day as starting dose [12]. Furthermore, a report from SJCRH in which 6MP dosages were adjusted to TPMT genotype showed no significant difference in relapse risks between patients with TPMTLA and TPMTWT [28]. Thus, the 6MP dosage strategy of NOPHO ALL2000 seemed successful in reducing the risk of SMN, but this effect was counteracted by relapse rates similar to that of patients with TPMTWT. The observed reduction in SMN primarily refers to the second hematologic malignancies since 80% of these occur within the first 5 years from diagnosis and furthermore seems

Fig. 4. Dose of 6-mercaptopurine (6MP) (A) and Methothrexate (MTX) (B) during maintenance therapy for patients with thiopurine methyltransferase low activity developing relapse or second malignant neoplasm (SMN) after start of maintenance therapy in NOPHO ALL92 and NOPHO ALL2000 studies. Day 0 is the day of diagnosis. A: 1 year: NOPHO ALL92 (n = 8); daily dose of 6MP, 60.3 mg/m² (54.1–68.9 mg/m²); NOPHO ALL2000 (n = 12); daily dose of 6MP, 44.5 mg/m² (32.9–55.7 mg/m²). Two years: NOPHO ALL92; daily dose of 6MP, 73.4 mg/m² (63.5–84.7 mg/m²); NOPHO ALL2000; daily dose of 6MP, 51.9 mg/m² (36.2–65.5 mg/m²). P-value for test of difference between curves = 0.12. B: 1 year: NOPHO ALL92 (n = 8); weekly dose of MTX, 14.9 mg/m² (12.8–16.6 mg/m²); NOPHO ALL2000 (n = 12); weekly dose of MTX, 14.6 mg/m² (11.8–16.8 mg/m²). Two years: NOPHO ALL92; weekly dose of MTX, 18.0 mg/m² (14.1–22.5 mg/m²); NOPHO ALL2000; weekly dose of MTX, 15.9 mg/m² (11.2–20.4 mg/m²). P-value for test of difference between curves = 0.78. 6MP indicates 6-mercaptopurine; and MTX, Methothrexate.

group, TPMT genotype had no effect on risk of relapse (TPMWT vs. TPMTLA hazard ratio (HR), 0.68 (0.37–1.26); P = 0.22).

The median 6MP starting dose for the 56 patients with TPMT heterozygosity was 50.0 mg/m²/day (50% range, 46.8–60.1 mg/m²/day). Seven patients received 6MP starting doses below 35 mg/m²/day due to leucopenia, neutropenia, or other previous toxicities, and seven patients initiated maintenance therapy with 6MP doses above 70 mg/m²/day due to high WBC at maintenance therapy onset or not taking TPMT status into account. For patients with TPMTLA who had their maintenance therapy starting dose of 6MP reduced to <75 mg/m²/day, the pSMN8y was 0%, whereas the pRELAPSE8y was 18.9% (10.9–32.3%).

The 6MP starting dose for patients with TPMTLA enrolled on ALL2000 was significantly lower than in ALL92 (median, 70 mg/ m²/day) (P < 0.0001). In addition, there was a trend towards patients with TPMTLA and event having a prescribed average lower dose of 6MP (median, 53.6 vs. 66.3 mg/m²/day; P = 0.045), but prescribed equal average MTX doses (median, 16.1 vs. 16.0 mg/m²/ week; P = 0.64) during the entire maintenance therapy when patients on ALL2000 and ALL92 were compared. Their smoothed maintenance therapy curves are depicted in Figure 4A and B. Neither the average WBC levels during maintenance therapy (median, 3.3 vs. 3.0 × 10⁹/L; P = 0.63) nor ANC levels (median, 1.8 vs. 1.6 × 10⁹/L; P = 0.92) differed significantly for the patients with TPMTLA and event in the ALL2000 versus ALL92 cohort.

DISCUSSION

Since the overall survival after childhood ALL has improved dramatically over the last decades, the proportional impact of death during remission including death after SMN has increased.

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connected to 6MP and MTX dose intensity and maintenance therapy duration [25]. Further follow-up will be needed to determine whether the reduced 6MP starting dose in the ALL2000 protocol will also affect the risk of the second cancers that occur later [29].

When comparing the other components of the NOPHO ALL92 and ALL2000 protocols, it seems unlikely that they explain our findings [14]. First, the overall cumulative incidences of relapse and SMN as well as the SMN incidences per risk group were almost identical in the two cohorts [11]. Second, the duration of maintenance therapy for patients in the IR group was increased from 2.0 to 2.5 years, which if anything should have reduced the relapse rate and increased not reduced the SMN risk [25,30]. Third, treatment with Erwinia asparaginase was substituted with E. coli asparaginase, but neither drug was given during maintenance therapy. Fourth, there is no difference between the previous and present study with respect to SMN pattern, since the most frequent type of SMN was therapy-related myeloid neoplasm in both cohorts. Fifth, if these changes in antileukemic therapy should explain the changes in risks of SMN and relapse for patients with TPMTLA, we would expect to see similar changes for patients with TPMTWT, but the risks of SMN and relapse for patients with TPMTWT do not differ in the two protocols.

The study patients in the ALL2000 and ALL92 cohorts are not completely comparable when it comes to the classification by TPMT. Thus, the majority of patients in the ALL92 cohort was only TPMT phenotyped during maintenance therapy and subsequently classified as presumed wild-type or heterozygous based on the trimodal distribution of TPMT activity [9,11], whereas in the present cohort only patients with available TPMT genotype were included. Thus, in the ALL92 study the TPMTLA group may have included a few patients with TPMTWT who had TPMT activity below the antimode of the TPMT phenotype distribution during maintenance therapy. However, if anything this would have weakened not strengthened the difference between patients with TPMTLA and TPMTWT with respect to risks of relapse and SMN.

In conclusion, the present study indicates that the reduced risk of SMN and the increased risk of relapse for patients with TPMTLA in the NOPHO ALL2000 cohort compared to the ALL92 cohort may be associated with reduction of 6MP starting dose to 50 mg/m²/day. Given the low relapse risk for patients with TPMTLA receiving starting 6MP doses of 75 mg/m² in NOPHO ALL92, the present study suggests that patients with TPMTLA or TPMTWT both should be treated with starting doses of 75 mg 6MP/m²/day. Since longer duration of therapy has been associated with SMN [25], one option could be to shorten the duration of maintenance therapy for patients with TPMTLA to 2 years as given in BFM protocols [12]. Further research is needed to identify patients with TPMTLA at excessive risk of SMN at standard 6MP doses to develop strategies for 6MP dose adjustments for such patients.

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REFERENCES