Intensification of Mercaptopurine/Methotrexate Maintenance Chemotherapy May Increase the Risk of Relapse for Some Children With Acute Lymphoblastic Leukemia

By Kjeld Schmiegelow, Olle Björk, Anders Glomstein, Göran Gustafsson, Niels Keiding, Jon Kristinsson, Anne Mäkipernaa, Susanne Rosthøj, Carol Szumlanski, Tine M Sørensen, and Richard Weinshilboum

Purpose: Thioguanine nucleotides (TGNs) mediate the cytotoxicity of mercaptopurine (MP). Methylated MP metabolites (formed by thiopurine methyltransferase [TPMT]) and methotrexate (MTX) polyglutamates can inhibit de novo purine synthesis. We explored whether dose adjustment of MP and MTX by erythrocyte (E) levels of TGN and MTX (including polyglutamates) could improve outcome in childhood acute lymphoblastic leukemia (ALL).

Patients and Methods: A total of 538 children with ALL were randomly assigned to have their oral MP/MTX maintenance therapy adjusted by white cell counts (WBC), E-TGN, and E-MTX (pharmacology group), or by WBC only (control group).

Results: After a median follow-up of 7.8 years, 79 patients had relapsed. Cox regression analysis showed an increased risk of relapse for boys (P = .0003), high WBC at diagnosis (P = .03), pharmacology arm (6.6 times increased relapse hazard for girls), high TPMT activity (P = .002), and high average neutrophil counts during maintenance therapy (P = .0009), with a significant interaction between sex and randomization group (P = .0007). For girls, the relapse risk was 5% in the control group and 19% in the pharmacology group (P = .001) because of an increased relapse hazard during the first year after cessation of therapy. TPMT activity was the most significant predictor of relapses among girls in the pharmacology arm (P < .0001). Overall, the TPMT activity was higher for patients who relapsed after cessation of therapy compared with those who stayed in remission (girls 19.5 v 17.4 U/mL, P = .03; boys 19.3 v 18.0 U/mL, P = .04).

Conclusion: Adding pharmacologically guided treatment intensification to dose adjustments by blood counts may not be warranted for girls, whereas new approaches to optimize maintenance therapy are needed for boys.

© 2003 by American Society of Clinical Oncology.
Randomization

Patients were to be randomly assigned to a regimen within 2 weeks before the start of maintenance therapy. The median number of days from randomization to the start of maintenance therapy was 9 days. The patients were randomly assigned to have their dose of MP and MTX adjusted by either blood counts (control group) or a combination of blood counts and E-TGN (pharmacology group). Patients were randomly assigned to these two groups with stratification for risk group (three divisions) and country (five divisions) within blocks of six patients. Thus, for each of these 15 subgroups defined by risk group and country, three patients were assigned to the control group and three patients to the pharmacology group for every six patients randomly assigned to a regimen.

Induction therapy consisted of prednisolone (60 mg/m²/day on days 1 to 36, then tapered), weekly vincristine (VCR; 2.0 mg/m² six times), doxorubicin (40 mg/m² three times [non-HR] or four times [HR]), Erwinia asparaginase (30,000 U/m² 10 times), and intrathecal MTX on four occasions. Consolidation therapy included high-dose MTX (HD-MTX, 5 g/m² three times) for SR-ALL, whereas patients with IR- and HR-ALL received alternating series of first, cyclophosphamide (total cumulative dose, 3 g/m²) with low-dose cytarabine and either oral MP or 6-TGN; second, HD-MTX (IR, 5 g/m² and HR, 8 g/m²; both administered four times) with either b-1 (IR only, oral MP [25 mg/m²/day] or b-2 (HR only, high-dose cytarabine [12 g/m² four times]) with two 2-month-interval periods of oral weekly MTX and daily MP with two VCR/prednisolone inductions per period; and third, 4 weeks of reinduction with dexamethasone (10 mg/m² for 3 weeks, then tapered), weekly VCR (2.0 mg/m² four times), weekly daunorubicin (30 mg/m²/d three times [HR] or four times [IR], and Erwinia asparaginase (30,000 U/m² four times).

Maintenance Therapy

Maintenance therapy with MP doses of 75 mg/m²/day and MTX doses of 20 mg/m²/wk was initiated 13 weeks (SR), 32 weeks (IR), or 63 weeks (HR) after diagnosis and continued until 2 years (IR and HR) or 2.5 years (SR) after diagnosis. During the first year of maintenance therapy, patients with SR- and IR-ALL received at 4-week intervals alternate pulses of either VCR (2.0 mg/m² once) and prednisolone (60 mg/m²/day for 1 week) or HD-MTX (5 g/m²) until five courses of HD-MTX had been given. Every 8 weeks throughout maintenance therapy, patients with HR-ALL received inductions of VCR (1.5 mg/m² once) and prednisolone (40 mg/m²/day for 1 week) with intrathecal MTX. HD-MTX 5- to 8-g courses during consolidation and maintenance therapy were given as 24-hour infusions with intrathecal MTX (age-adjusted doses) and leucovorin rescue from 36 hours to be continued at 6-hour intervals until plasma-MTX was less than 200 nmol/L.

Abbreviations: SR, standard risk; IR, intermediate risk; HR, high risk; ALL, acute lymphoblastic leukemia; BM, bone marrow, MT, maintenance therapy; CCR, complete continuous remission; DK/IF/N/S, Denmark, Iceland, Finland, Norway, Sweden; VHR, very high risk; NOPHO, Nordic Society of Paediatric Haematology and Oncology.

*Age, 2.0-9.9 years and WBC < 10 × 10⁹/L; no HR criteria.
†Age, 1.0-1.9 years or > 10.0 years and/or WBC 10-49 × 10⁹/L; no HR criteria.
‡WBC ≥ 50 × 10⁹/L; T-cell, mediastinal; CNS, testicular, or lymphomatous disease, t(4;11), t(9;22), or 22q-, and M3 d14 or M2/3 d29 BM.
§1.0-1.9 years, diagnosed in 1992-1996 with non-B non-VHR ALL (and not Finish HR-ALL), and starting maintenance therapy before January 1, 1997. VHR status is assigned if age ≥ 5 years and, in addition, T-cell disease is present with one or more additional HR features, CNS leukemia, lymphomatous leukemia, or HR-ALL criteria at diagnosis, and a day 15 M3 or a day 29 M2/3 bone marrow.
(Median age or WBC at diagnosis (75% range).)
MP/MITX Dose Adjustments: Control Group

The doses of oral MP and MTX were targeted to a WBC of 1.5 to 3.5 × 10^9/L; reductions to 50% were recommended at a WBC less than 1.5 × 10^9/L, and therapy was interrupted at a WBC less than 1.0 × 10^9/L or a thrombocyte count less than 100 × 10^9/L. Therapy was reinitiated when blood counts were increasing and WBC was more than 1.5 × 10^9/L. Blood counts were measured at an average interval of 1 to 2 weeks. If WBC was more than 3.5 × 10^9/L, the protocol recommended upward dose adjustments of MTX and/or MP until the WBC was within the target range.

MP/MITX Dose Adjustments: Pharmacology Group

The doses of oral MP and oral MTX were targeted by WBC and thrombocyte counts as were those for the control group. In addition and unless the WBC was less than 1.5 × 10^9/L, the doses of MP and/or MTX were to be adjusted upward in steps of 20% if E-TGN × MTX was less than 1,350 (nmol/mmol hemoglobin [Hb])^2 and the treating physician regarded such dose adjustments to be tolerable. This E-TGN × MTX value represents a target level of 225 nmol/mm Hb for E-TGN and 6.0 nmol/mmol Hb for E-MTX; these values are approximately 25% above the median levels that discriminated between patients with a good or a worse prognosis in the NOPHO ALL-88 study.2 Because the published data on the relation of relapse risk were stronger for E-TGN than for E-MTX, dose adjustments of MP were made before adjustments of MTX until the E-TGN was more than 225 nmol/mm Hb. However, if this value was not achieved within 8 weeks from the time of recommended adjustments, patients were to have upward dose adjustments for MP and/or MTX at the discretion of the treating physician. Because interim analyses in July 1998 (1.5 years after patient accrual had stopped) strongly indicated an increased risk of relapse for patients in the pharmacology group, pharmacologic-based dose titration was stopped and all patients still receiving maintenance therapy were thereafter adjusted by the blood counts only. At that time point, only 13 of the 24 SR-ALL patients in the pharmacology group who were still receiving therapy had more than 3 months of their therapy remaining (maximum, 8 months). Thus far, only one of those 24 patients has developed a relapse.

E-TGN/MITX and TPMT

At least once a month, unfrozen blood samples were to be sent for E-TGN/MITX analyses at the Laboratory for Pediatric Oncology (Bonkolab), H.S Rigshospitalet, Copenhagen.17,18 If no blood samples were received for 6 weeks, a reminder was sent out. If the blood sample was too small to allow analyses of both E-TGN and E-MTX, these analyses were done alternately. The time of shipment exceeded 5 days for 501 samples (5.0%), and those results were excluded from the statistical analyses because E-TGN and E-MTX are only stable in unfrozen blood for up to 5 days (K. Schmiegelow, unpublished data). If oral MTX had been taken less than 48 hours or HDM had been administered less than 1 week before blood sampling, recommendations with regard to MTX dose adjustments were based on the previous E-MTX value unless the E-MTX in question was below 6.0 nmol/mmol Hb and E-TGN × MTX was less than 1,350 (nmol/mmol Hb). However, irrespective of their levels, such E-MTX values were excluded from statistical analysis of the relation between E-MTX levels and outcome. For 507 of 538 patients, the RBC TPMT activity was measured one to five times during maintenance therapy as previously described.19 All TPMT assays were performed at least 8 weeks after the most recent blood transfusion. For 344 of these patients, erythrocyte levels of methylated MP metabolites were measured together with TPMT. Because of the low number of measurements (median, 1; 75% range, 1 to 3), these data were not included in the statistical analyses.

Statistical Methods

For patients with more than one TPMT measurement, an arithmetic mean TPMT was calculated and TPMT was considered a fixed covariate. Non-parametric methods were applied to compare the distribution of parameters between subgroups.20 In these comparisons, patients in remission did not include those who died in remission or who developed a second malignancy. Cox proportional hazards backward regression analyses were performed, and the likelihood-ratio test was applied to test for differences in outcome.21 Covariates were excluded from the models at a significance level of 0.05.

Randomization group (pharmacology v control group), E-TGN, and E-MTX levels were tested as interacting covariates with respect to sex.23 Where relevant, the covariates were analyzed as time-dependent continuous parameters with recalculations of the weighted means (prefix m) of these variables every time a patient treatment failed using as weight the interval between the sample in question and the next blood sample.5 Because the background relapse intensity differed among the risk groups, the Cox analyses were stratified for risk groups assuming the same effect (ie, hazard coefficient) of possible prognostic factors across risk groups.22 Survival analyses were done with a basic time scale defined by the date of diagnosis with delayed entry of patients at the start of maintenance therapy, or at the time of the first E-TGN/MITX or blood count measurements after initiation of maintenance therapy if the survival analyses included such time-dependent parameters.22 As events in the event-free survival (EFS) analyses, we included relapse, death in remission, or the diagnosis of a second malignant neoplasm, whichever occurred first. Patients who died in first remission or who developed a second cancer were censored at the time of these events in the analysis of relapse risk factors. The Kaplan-Meier method was applied for estimation of remission duration and for the generation of survival curves.24 Subgroups were compared with the log-rank test25 and were stratified where needed. Two-sided values of P < .05 were regarded as being significant. Survival analyses were performed with the SAS (SAS Institute, Cary, NC) or S-plus (Statistical Sciences, Seattle, WA) statistical software.26

The protocol was approved by the ethical committee of Copenhagen (no. V.200.2080/91) as well as by the local ethical committees, and participants gave informed consent according to the Helsinki Declaration.

RESULTS

The patients were followed until December 31, 2001. For patients who stayed in remission, the median length of follow-up from diagnosis was 93 months (50% range, 80 to 106 months). The EFS for 9 years (EFS_9y) for all children with non-B ALL and aged 1.0 to 14.9 years when diagnosed in the Nordic countries during the study period from 1992 to 2000 was 0.75 ± 0.01. Because patients who developed an event or were transplanted before the start of maintenance therapy were not eligible for the study, the EFS_9y for the 538 patients who entered the study was slightly better than 0.75: 0.83 ± 0.02 (SR, 0.85 ± 0.03; IR, 0.81 ± 0.03; HR, 0.79 ± 0.07).

Relapse Rate

Seventy-nine of the 538 study patients (45 in the pharmacology group) developed a relapse (93% of all relapses projected at 9 years) at a median of 38 months after diagnosis (range, 11 to 101 months), 66 of which occurred after the cessation of therapy (Table 2). Three patients died in first remission during maintenance therapy (two patients in the control group), and seven patients (three patients in the control group) developed a second cancer (one malignant brain tumor and six myelodysplasias or acute myeloid leukemias) 23 to 63 months after diagnosis.27 Figure 1 demonstrates that for SR and IR patients, the relapse hazard peaks within the first year after cessation of therapy.

We used Cox multivariate regression analysis of all 538 patients to test the effect on relapse risk of year of diagnosis (0 for 1992 to 1993 v 1 for 1994 to 1996), sex, WBC at diagnosis, age at diagnosis, and randomization group. Randomization group, sex, and the interaction between these variables were significantly correlated with relapse risk (overall value of the Cox model, P = .001). Subsequently, we included the following time-dependent covariates in the Cox analysis: TPMT activity, the average dose of MTX and MP, the percentage of mainte-
nance therapy with treatment discontinuation of MP and MTX, weighted mean of the absolute neutrophil count (mANC), E-TGN, E-MTX, and the proportion of maintenance therapy with E-TGN, E-MTX, and mE-TGN/Hb above 225 nmol/ 6.0 nmol/mmol Hb, and 1,350 (nmol/mmol Hb), respectively. The overall best-fit Cox model to predict risk of relapse included WBC at diagnosis, randomization group, sex (the latter two as interacting variables), TPMT activity, and mANC (Table 3). Forty-nine patients (including seven patients with a relapse) had to be excluded because they lacked TPMT activity (n = 31) or neutrophil count measurements (n = 18). Except for WBC at diagnosis, the coefficients of the significant variables in Table 3 changed less than 1% if TPMT activity was included in the Cox analysis as a dichotomous variable (or 1/14 U/mL).

E-TGN levels and TPMT activity were closely related. Thus, if the TPMT activity was excluded from the Cox analysis, E-TGN levels were borderline predictive of the risk of relapse ($\beta = -0.0023$, $P = .07$). Because boys did not seem to gain from pharmacological dose adjustments, and because the relapse hazard for girls randomly assigned to the pharmacology group was increased 6.6 times compared with that of the control group (9-year cumulative relapse risk, 19% ± 5% vs 5% ± 2%; $P = .001$; Table 3; Fig 2), we performed additional analyses of drug dosage and pharmacokinetics to explore further the results.

**Dose of MTX and MP**

Boys received a significantly higher average dose of both MTX (median, 16.2 vs 14.6 mg/m²; $P = .0007$) and of MP (median, 61.3 vs 57.2 mg/m²; $P = .01$) than did girls. Patients in the pharmacology and control groups did not differ significantly in their average doses of MTX and MP, but if we included in the analyses only the periods during which MP was administered, boys in the pharmacology group received a higher average dose of MP compared with boys in the control arm (median, 68.5 vs 63.8 mg/m²; $P = .05$), whereas there was no significant difference for girls (median, 62.1 vs 63.8 mg/m²; $P = .85$). Conversely, girls (but not boys) in the pharmacology group were

<table>
<thead>
<tr>
<th>Table 2. Distribution of Events by Sex, Risk Group, and Randomization Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Intermediate risk</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>High risk</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Intermediate risk</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>High risk</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: CCR, complete continuous remission.
*Patients in first remission December 31, 2002.
†Second cancers include one malignant brain tumor and six myelodysplasias or acute myeloid leukemias.
registered with significantly more cumulative MP treatment interruptions than were those in the control group (median for girls, 8% vs 5% of the total duration of maintenance therapy; \( P = .01 \) compared with median for boys, 6% vs 5%; \( P = .29 \)). Overall, patients who relapsed did not differ in their average dose of MP or MTX from those who stayed in remission, but compared with patients who stayed in remission, the 13 patients who relapsed while receiving therapy (10 of which were boys) received lower doses of MTX (median, 12.6 vs 15.2 mg/m\(^2\); \( P = .01 \)) and of MP (49. vs 59.3 mg/m\(^2\); \( P = .06 \)).

**TPMT Activity**

Of the 507 patients for whom TPMT measurements were available, two were TPMT deficient (TPMT\(^{-/}L\), TPMT < 1.0 U/mL RBC), and 72 (14%) were classified as heterozygous (TPMT\(^{+/}L\), TPMT < 14.0 U/mL RBC, antimode of the bimodal distribution). The TPMT activity did not differ when the control group was compared with the pharmacology group (median for girls, 17.6 vs 17.6 U/mL; \( P = 1.00 \) compared with median for boys, 18.3 vs 18.2 U/mL; \( P = .20 \)). Compared with patients with TPMT more than or equal to 14 U/mL, those with TPMT activity less than 14 U/mL received a lower average dose of MP (median, 52.4 vs 61.5 mg/m\(^2\); \( P = .0001 \)), experienced more treatment interruptions (median, 9% vs 5%; \( P = .003 \)), had higher mE-TGN levels (median, 334 vs 157 nmol/mmol Hb; \( P = .0001 \)), and experienced lower mWBC levels (median, 3.1 vs 3.4 \( \times 10^9 /L \); \( P = .02 \)). The 62 patients who relapsed after cessation of therapy and who had TPMT measurements available had significantly

### Table 3. Coefficients in the Cox Hazard Models: Relapses Only*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta</th>
<th>SE, ( \beta )</th>
<th>Relapse Hazard</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex†</td>
<td>1.99</td>
<td>0.61</td>
<td>7.3</td>
<td>.0003</td>
</tr>
<tr>
<td>WBC at diagnosis</td>
<td>5.61 ( \times 10^{-3} )</td>
<td>2.26 ( \times 10^{-3} )</td>
<td>1.01</td>
<td>.03</td>
</tr>
<tr>
<td>Randomization group‡</td>
<td>1.89</td>
<td>0.620</td>
<td>6.6</td>
<td>.0003</td>
</tr>
<tr>
<td>Randomization group × sex,§</td>
<td>−2.02</td>
<td>0.684</td>
<td>0.13</td>
<td>.0007</td>
</tr>
<tr>
<td>mANC</td>
<td></td>
<td>0.609</td>
<td>0.175</td>
<td>1.83</td>
</tr>
<tr>
<td>TPMT activity</td>
<td>0.101</td>
<td>0.033</td>
<td>1.1</td>
<td>.002</td>
</tr>
</tbody>
</table>

Abbreviations: mANC, mean absolute neutrophil count; TPMT, thiopurine methyltransferase.

*Death in remission and second cancers were counted as censoring events.
†0 for girls, 1 for boys.
‡0 for control, 1 for pharmacology.
§The interaction between sex and randomization group, 1 for boys in pharmacology group, 0 for all other.
| Mean absolute neutrophil count during maintenance therapy was analyzed as a time-dependent continuous variable. Overall \( P \) value of the Cox model < 0.0001."
higher TPMT levels than those who stayed in remission (median for girls, 19.5 ± 17.4 U/mL; \( P = .03 \) compared with median for boys, 19.3 ± 18.0 U/mL; \( P = .04 \)). In contrast, this was not the case for those 12 patients with TPMT measurements who relapsed while receiving therapy (\( P = .61 \)). In a multivariate analysis, the best-fit model to predict the risk of relapse after cessation of therapy among girls in the pharmacology group (17 relapses) included mANC (\( \beta = 0.93; P = .002 \)) and the TPMT activity (\( \beta = 0.15; P < .0001 \)). Not included in the model were WBC at diagnosis, average dose of MTX and MP, and cumulative duration of treatment interruptions of MTX and/or MP, mE-TGN, and mE-MTX.

### E-TGN and E-MTX

The median number of E-TGN/MTX blood samples analyzed for patients in first remission at the end of maintenance therapy and for patients who relapsed during maintenance therapy was 16 and 12 (75% range, 7 to 30 and 4 to 22 samples), respectively (9,182 TGN/MTX measurements in total). When patients who stayed in remission were analyzed, girls and boys did not differ significantly in their mE-TGN levels (median, 169 ± 169 nmol/mmol Hb; \( P = .31 \)), but boys had higher mE-MTX levels (median for girls, 5.4 and median for boys, 5.8 nmol/mmol Hb; \( P = .01 \)) and mE-TGN × MTX levels (1,087 ± 896 nmol/mmol Hb; \( P = .001 \)). The two treatment arms did not differ in their m-E-TGN levels (\( P = .63 \)), m-E-MTX levels (\( P = .71 \)), or m-E-TGN × MTX levels (\( P = .55 \)). Patients who developed a relapse while receiving therapy had lower m-E-TGN levels than those who have stayed in remission (104 ± 169; \( P = .005 \)), although this was not the case for those who relapsed after cessation of therapy (165 ± 169 nmol/mmol Hb; \( P = .63 \)). Neither m-E-MTX nor m-E-TGN × MTX levels differed significantly between the patients who relapsed and those who stayed in remission (\( P = .13 \) and \( P = .76 \), respectively).

### Blood Counts

A total of 28,580 data sets on blood counts and/or drug doses were included in the analyses. For patients who stayed in remission, the mWBC levels did not differ significantly between boys and girls (median, 3.3 ± 10^9/L for both sexes; \( P = .59 \)). Compared with those in the control group, patients in the pharmacology group had lower mANC (1.9 ± 2.0 ± 10^9/L; \( P = .04 \)) but did not differ significantly in mWBC (3.2 ± 3.4 ± 10^9/L; \( P = .22 \)). The patients who relapsed had significantly higher mANC levels than those who stayed in remission (median, 2.2 v 1.9 ± 10^9/L; \( P = .0008 \)) but did not differ significantly in their mWBC (3.5 ± 3.3 ± 10^9/L; \( P = .06 \)), average lymphocyte count levels (\( P = .60 \)), or median monthly number of blood counts (2.9 v 2.5; \( P = .13 \)). For both sexes and without adjustments for possible confounders, patients with a mANC less than 2.0 ± 10^9/L (median of all patients) had an outcome superior to that of patients with higher mANC levels (for boys, 0.87 v 0.75, \( P = .02 \); and for girls, 0.94 v 0.83, \( P = .01 \)).

### DISCUSSION

Treatment intensity during maintenance therapy reflects the dose adjustment guidelines of the protocol and the physician and patient compliance.\(^2\) In retrospect, the clinical effect of these factors is difficult to separate. Thus, the results of this study do not necessarily imply that dose adjustments to achieve a certain degree of systemic drug exposure during maintenance therapy will not improve cure rate. However, in contrast to the aims of the study, pharmacologically guided dose adjustments failed to improve the outcome for boys but significantly increased the relapse risk for girls. These data seem counterintuitive, but they carry important information that may expand our understanding of the way in which maintenance therapy works. Because this and previous studies indicate important sex differences in the course of childhood ALL, the results for boys and girls will be discussed separately.\(^2\)\(^3\)\(^0\)\(^3\)\(^2\)

For boys, the NOPHO ALL-88 study and other cohorts\(^1\)\(^2\)\(^3\)\(^3\) have found that E-TGN levels are related to the cure rate, although this was not the case in a recent study by Relling et al.\(^4\) In this study, E-TGN levels were only of significance for patients who relapsed while receiving therapy. Thus, the divergent results of these studies could partly reflect the distribution of relapses while patients were receiving and not receiving therapy. In addition, E-TGN levels are surrogate markers for TPMT activity, and their prognostic significance probably reflects the effect on MP pharmacology by TPMT. Although TPMT activity is related to E-TGN, and erythrocyte and lymphoblast TPMT activities are closely related,\(^3\) E-TGN levels will only be reflections for events in leukemic blasts, for which the end point metabolites are not cytosol TGN but rather DNA-TGN.\(^5\) It is also likely that other MP metabolites such as methylthioinosine monophosphate (formed by TPMT) could influence the degree of DNA-TGN incorporation and cytotoxicity through inhibition of de novo purine synthesis.\(^1\)\(^2\)\(^3\)\(^6\)

Finally, several studies have indicated that merely securing high E-TGN levels may be insufficient to reduce the risk of relapse. First, United Kingdom ALL Study Group (UKALL) and Cooperative ALL Study Group (COALL) protocols have randomly assigned patients to either MP or thioguanine (TG) as part of their maintenance therapy without significant reductions in relapse rates.\(^3\)\(^7\)\(^3\)\(^8\) Although the patients in the COALL 05–92 study who were given TG did achieve high E-TGN levels, they did not experience significantly more leukopenia and did not achieve a significant reduction in relapse rate.\(^3\)\(^8\)\(^4\)\(^0\) Second, patients with low TPMT activity, not least who that are TPMT deficient, can tolerate two- to 10-fold higher RBC cytosol TGN levels than can patients with a wild-type TPMT genotype and phenotype, which also indicates that cytosol TGN concentrations are not directly reflected in the DNA-TGN levels.\(^3\)\(^9\)\(^4\)\(^1\) Thus, even if low E-TGN levels are markers for inferior treatment intensity of boys, the ideal way in which to compensate for this remains to be determined.

The cause of the significantly increased relapse rate experienced by the girls in the pharmacology group within the first year off therapy is unclear. Dose increments could have increased suppression of immune functions that play a role in the eradication of the leukemic clone. However, apart from the graft-versus-leukemia phenomenon observed after stem-cell transplantation, no clinical studies have demonstrated that the immune system plays a substantial role in the cure of childhood ALL. In addition, lymphocyte counts did not differ significantly between the control and pharmacology groups or between patients who...
experienced a relapse and those who remained in remission (data not shown). Both this study and previous studies have shown that attempts to escalate the dose of MP are counteracted by longer periods of therapy withdrawal and may, in addition, preferentially increase the levels of methylated metabolites. As the overall relapse risk has been reduced, the increased risk of relapse within the first year after the cessation of maintenance therapy has become obvious. Thus, even though contemporary treatment intensity has reduced the risk of treatment failures, the relapse rate following cessation of therapy seems to have changed little.

In this study, TPMT activity was the strongest treatment-related parameter with influence on the risk of relapse among girls in the pharmacology group. This could indicate that attempts to escalate the dose of MP lead to increased intracellular levels of methylated MP metabolites, with significant inhibition of purine de novo synthesis in the leukemic lymphoblasts for some patients with high TPMT and a delay of leukemic cells in S phase, putting these cells into a dormant state with regrowth after treatment discontinuation. The theory of dormant leukemia with intensified maintenance therapy is indirectly supported by the Japanese Children’s Cancer and Leukemia Study Group-S811 study, which demonstrated that intermittent MP/MTX therapy (possibly allowing intermittent leukemic regrowth and thus susceptibility to MP/MTX therapy) was superior to continuous MP/MTX therapy. Similarly, the Dutch ALL-6 protocol gave an excellent EFS (0.81 at 8 years) for patients with low-risk ALL (WBC < 50 × 10⁹/L at diagnosis) that could be in part because of the use of intermittent MTX/MP maintenance therapy with 2 weeks interruption of oral MTX/MP during every 7-week cycle. Although the UKALL studies in the early 1980s indicated that continuous therapy was superior to intermittent therapy, the overall outcome at that time was generally poor, and whether continuity of therapy or physician compliance with the protocol had the major effect on the improvement of outcome is not known.

That mE-MTX was not significantly related to the overall risk of relapse (as in the NOPHO ALL-88 study) could reflect, in part, that the dose of intravenous (IV) MTX had been increased from 0.5 to 1 g/m² in the ALL-88 protocol to 5.0 to 8.0 g/m² in the ALL-92 protocol, and this could have mediated the major antileukemic effect of MTX. However, measuring MTX/folate ratios may be superior to intracellular MTX levels, and further research is needed to identify the optimal way by which to monitor and MTX therapy.

The relation between mANC and the risk of relapse, and the excellent outcome for girls in the control group, indicate that dose adjustments on the basis of blood counts are sufficient to optimize therapy for girls and that unnecessary treatment intensification could upset their favorable outcome. For boys, new approaches for the optimization of maintenance therapy are needed. In future trials, the value of the monitoring levels of methylated and nonmethylated MP metabolites and DNA-TGN may increase our understanding of the pharmacokinetic and pharmacodynamic background for treatment failures.

ACKNOWLEDGMENT

The commitment and skilful technical assistance of Line Merrild Brixen, Jannie Gregers, Kristine Nielsen, Anne Sørensen, Michael Timm, and Mads Tvede are greatly appreciated.

APPENDIX

The appendix is available online at www.jco.org.

REFERENCES


