

blood

2009 113: 6077-6084
Prepublished online February 17, 2009;
doi:10.1182/blood-2008-11-187880

Methotrexate/6-mercaptopurine maintenance therapy influences the risk of a second malignant neoplasm after childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study

Kjeld Schmiegelow, Ibrahim Al-Modhwahi, Mette Klarskov Andersen, Mikael Behrendtz, Erik Forestier, Henrik Hasle, Mats Heyman, Jon Kristinsson, Jacob Nersting, Randi Nygaard, Anne Louise Svendsen, Kim Vettenranta and Richard Weinshilboum

Updated information and services can be found at:
<http://bloodjournal.hematologylibrary.org/content/113/24/6077.full.html>

Articles on similar topics can be found in the following Blood collections
[Clinical Trials and Observations](#) (3763 articles)
[Free Research Articles](#) (1911 articles)
[Lymphoid Neoplasia](#) (1499 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://bloodjournal.hematologylibrary.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
<http://bloodjournal.hematologylibrary.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:
<http://bloodjournal.hematologylibrary.org/site/subscriptions/index.xhtml>



Methotrexate/6-mercaptopurine maintenance therapy influences the risk of a second malignant neoplasm after childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study

Kjeld Schmiegelow,^{1,2} Ibrahim Al-Modhwah,² Mette Klarskov Andersen,³ Mikael Behrendtz,⁴ Erik Forestier,⁵ Henrik Hasle,⁶ Mats Heyman,⁷ Jon Kristinsson,⁸ Jacob Nersting,² Randi Nygaard,⁹ Anne Louise Svendsen,¹⁰ Kim Vetterranta,¹¹ and Richard Weinshilboum,¹² for the Nordic Society for Paediatric Haematology and Oncology

¹Faculty of Medicine, Institute of Gynecology, Obstetrics, and Pediatrics, University of Copenhagen, Copenhagen, Denmark; ²Department of Pediatrics and ³The Cytogenetic Laboratory, The University Hospital Rigshospitalet, Copenhagen, Denmark; ⁴Department of Pediatrics, The University Hospital Linköping, Stockholm, Sweden; ⁵Department of Pediatrics, The University Hospital Umeå, Stockholm, Sweden; ⁶Department of Pediatrics, Aarhus University Hospital, Skejby, Denmark; ⁷Department of Pediatrics, Astrid Lindgrens Barnsjukhus, Stockholm, Sweden; ⁸Department of Pediatrics, University Hospital, Reykjavik, Iceland; ⁹Department of Pediatrics, University Hospital, Trondheim, Norway; ¹⁰Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark; ¹¹Department of Pediatrics, University Hospital, Helsinki, Finland; and ¹²Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, MN

Among 1614 children with acute lymphoblastic leukemia (ALL) treated with the Nordic Society for Paediatric Haematology and Oncology (NOPHO) ALL-92 protocol, 20 patients developed a second malignant neoplasm (SMN) with a cumulative risk of 1.6% at 12 years from the diagnosis of ALL. Nine of the 16 acute myeloid leukemias or myelodysplastic syndromes had monosomy 7 (n = 7) or 7q deletions (n = 2). In Cox multivariate analysis, longer duration of oral 6-mercaptopurine

(6MP)/methotrexate (MTX) maintenance therapy (P = .02; longest for standard-risk patients) and presence of high hyperdiploidy (P = .07) were related to increased risk of SMN. Thiopurine methyltransferase (TPMT) methylates 6MP and its metabolites, and thus reduces cellular levels of cytotoxic 6-thioguanine nucleotides. Of 524 patients who had erythrocyte TPMT activity measured, the median TPMT activity in 9 patients developing an SMN was significantly lower than in the

515 that did not develop an SMN (median, 12.1 vs 18.1 IU/mL; P = .02). Among 427 TPMT wild-type patients for whom the 6MP dose was registered, those who developed SMN received higher average 6MP doses than the remaining patients (69.7 vs 60.4 mg/m²; P = .03). This study indicates that the duration and intensity of 6MP/MTX maintenance therapy of childhood ALL may influence the risk of SMNs in childhood ALL. (Blood. 2009;113:6077-6084)

Introduction

A few percent of children with acute lymphoblastic leukemia (ALL) will develop a second malignant neoplasm (SMN), and this complication has a poor prognosis.¹⁻⁵ Treatment-related risk factor analyses have focused primarily on irradiation, alkylating agents, and topoisomerase II inhibitors such as anthracyclines and epipodophyllotoxins that can induce DNA damage, whereas less attention has been paid to mechanisms that could interfere with DNA control, including drugs that may modify DNA repair.⁵

Previously, children with higher risk ALL had a higher risk of SMNs because of their more intensive chemotherapy, radiotherapy, or both.⁶ However, a few studies have indicated that the less intensive 6-mercaptopurine (6MP)/methotrexate (MTX) maintenance therapy also enhances the risk of SMNs. The Nordic Society for Paediatric Haematology and Oncology (NOPHO) ALL-92 6MP/MTX maintenance therapy trial reported 3 cases of acute myeloid leukemia or myelodysplastic syndrome (t-AML/MDS) among 55 children, who had thiopurine methyltransferase (TPMT) activity compatible with TPMT low-activity polymorphisms (cumulative risk, 9%), compared

with only 2 cases among 384 patients (cumulative risk, 1%) with normal TPMT activity.⁷ Similarly, St Jude Children's Research Hospital has reported that children with ALL who were heterozygous for TPMT low-activity polymorphisms had an excessive risk of SMNs if exposed to cranial irradiation.⁸ Low-activity TPMT polymorphisms lead to higher intracellular levels of 6-thioguanine nucleotides (6TGN), which are the cytotoxic 6MP metabolites that are incorporated into DNA.^{9,10} In addition, some of the methylated 6MP metabolites formed by TPMT are strong inhibitors of purine de novo synthesis,¹¹ and, because their levels increase with higher doses of 6MP,¹² such 6MP dose increments in TPMT high-activity patients may lead to enhanced DNA-6TGN incorporation even in patients without TPMT low-activity polymorphisms.

To explore further the possible effect of 6MP/MTX maintenance therapy on the risk of SMNs, we reviewed the cumulative incidence of SMNs among the total cohort of 1614 patients treated according to the NOPHO ALL-92 protocol and report that the risk of SMNs in patients treated on this protocol correlates with TPMT

Submitted November 5, 2008; accepted January 22, 2009. Prepublished online as *Blood* First Edition paper, February 17, 2009; DOI 10.1182/blood-2008-11-187880.

An Inside *Blood* analysis of this article appears at the front of this issue.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2009 by The American Society of Hematology

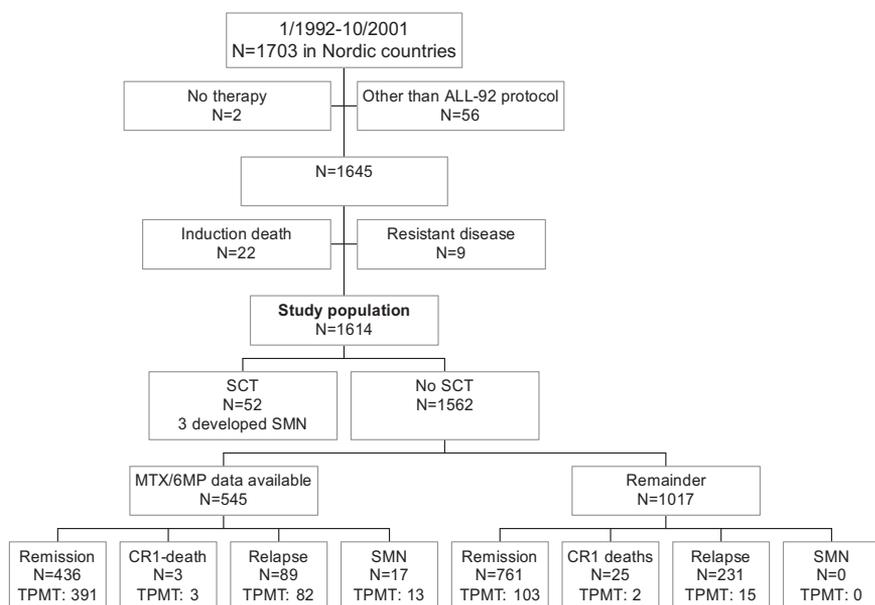


Figure 1. Flow chart of patient inclusion or exclusion. Patients (n = 545) participated in the randomized MTX/6MP maintenance therapy study¹⁶ or they developed SMNs and had their data on maintenance therapy 6MP/MTX dosage and blood counts retrieved. Data on both 6MP/MTX doses and blood counts were not available for all the 545 patients. TPMT phenotyping (n = 524) or genotyping or both were available for 609 patients.

activity or the dose intensity of oral 6MP/MTX maintenance therapy or both.

Methods

Patients

From January 1992 until October 2001, 1703 children 1.0 to 14.9 years of age were diagnosed with B-cell precursor or T-cell ALL in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden; Figure 1). Two patients with Down syndrome received no antileukemic therapy, 10 patients were treated according to non-NOPHO ALL protocols, 1 patient was treated according to the previous NOPHO ALL-86 protocol, and 45 patients were treated according to the NOPHO ALL-2000 protocol, before it was officially opened. None of these 58 patients have developed an SMN during their first complete remission (CR1). Of the remaining 1645 patients who started therapy according to the NOPHO ALL-92 protocol, 22 patients died during induction therapy, and 9 patients had resistant disease and were

excluded from further analysis. The remaining 875 boys and 739 girls were included in the present study. Twenty-nine of these 1614 patients had Down syndrome. The data used in this publication were retrieved from the NOPHO Leukemia Registry. This registration has been approved by the relevant ethical committees and the data protection agencies in the involved countries. Informed consent was obtained in accordance with the Declaration of Helsinki.

Risk grouping

The risk group criteria are given in Table 1.¹³ The patients who had higher risk features at diagnosis and were assigned to the very high risk (VHR) treatment arm received prophylactic or therapeutic central nervous system (CNS) irradiation as well as LSA₂L₂ instead of MTX/6MP maintenance therapy.

Treatment

For 55 of the 1614 patients, the assigned treatment did not completely match the risk group criteria. Thirty-five patients with morphologically

Table 1. Risk group, therapy, and second malignant neoplasms in NOPHO ALL-92 protocol

	SR-ALL (n = 562)*	IR-ALL (n = 590)†	HR-ALL (n = 239)‡	VHR-ALL (n = 223)§
Doxorubicin/daunomycin	120	240	280	400
Cyclophosphamide	0	3,000	3,000	6,600
Cranial irradiation	No	No	No	Yes
6MP/MTX (weeks)	117	72	41	0-8
Dead in CR1/relapse/SMN¶	7/85/12	9/106/4	8/80/2	10/64/2
pEFS/pOS¶	0.81/0.90	0.79/0.90	0.62/0.74	0.66/0.74
AML/MDS/solid tumor	5/7/0	1/1/2	2#/0/0	0/0/2#¶
12-year pSMN, mean ± SE (%)**	2.4 ± 0.7	1.2 ± 0.7	1.2 ± 0.8	1.1 ± 0.8

AML indicates acute myeloid leukemia; CNS, central nervous system; dead in CR1, dead in first remission; M2/3 BM, greater than 5%/25% leukemic blasts in bone marrow; MDS, myelodysplastic syndrome; 6MP/MTX, duration of oral 6MP/MTX maintenance therapy in weeks; pEFS/pOS, the 12-year probability of event-free survival and overall survival, respectively; SMN, second malignant neoplasm; SR/IR/HR/VHR, standard/intermediate/high/very high risk of acute lymphoblastic leukemia; and WBC, white blood cell.

*Risk criteria were age of 2.0 to 9.9 years, WBC count less than 10 × 10⁹/L, and no HR/VHR criteria.

†Risk criteria were age of 1.0 to 1.9 year (or older than 10.0 years) or WBC count of 10 to 49 × 10⁹/L, and no HR/VHR criteria.

‡Risk criteria were WBC count at least 50 × 10⁹/L; T-cell, mediastinal, CNS, testicular, or lymphomatous disease; t(4;11) or t(9;22); M3 d15 or M2/3 d29; and no VHR criteria.

§Risk criteria were HR-ALL at diagnosis and at least 5 years of age and (1) CNS leukemia, or (2) lymphomatous leukemia, or (3) d15 M3 or a d29 M2/3 BM, or (4) T-ALL with 1 or more other HR features.

||Cumulative dose in mg/m².

¶Twelve-year probability of event-free survival and overall survival.

#Three of the 4 SMNs occurred after stem cell transplantation in first remission.

**Twelve-year probability of developing an SMN.

suspected, but uncertain, M3 bone marrow on day 15 or M2 bone marrow on day 29 or both had standard risk (SR)-ALL ($n = 20$) or intermediate risk (IR)-ALL ($n = 15$) by all other criteria and were treated according to the SR or IR treatment arms, respectively. Two patients who fulfilled the protocol criteria for SR-ALL or IR-ALL were by decision of the treating physician assigned to the VHR-arm because of a $t(1;19)(q23;p13)$ translocation ($n = 1$) or hypodiploidy ($n = 1$; modal number 35-36). Three patients who fulfilled the VHR-ALL criteria were by decision of the treating physician assigned to the high-risk (HR) arm. Furthermore, 9 patients who fulfilled the SR-ALL criteria were by decision of the treating physician assigned to the IR arm, and 6 patients who fulfilled the IR-ALL criteria were likewise assigned to the SR arm. None of those 55 patients have developed an SMN, and all were analyzed according to the therapy they actually received.

Induction therapy and consolidation therapy. These therapies have previously been published in detail.¹³ In short, all patients received prednisolone (60 mg/m² per day on days 1-36, then tapered), weekly vincristine (VCR; 2.0 mg/m² times 6), doxorubicin (40 mg/m² times 3 [SR and IR] or 4 [HR and VHR]), *Erwinia* asparaginase (30,000 IU/m² daily on days 37-46), and intrathecal MTX on 4 occasions. Subsequently and before MT, SR-ALL patients received 3 courses of high-dose MTX (HD-MTX; 5 g/m² every 24 hours with intrathecal MTX and leucovorin rescue), whereas patients with IR-, HR-, and VHR-ALL received alternating combination therapy blocks.

CNS irradiation and stem cell transplantation. Because of CNS disease at diagnosis or allocation to the VHR protocol arm, 123 patients who did not receive a transplant (7.6% of the total study population) received cranial irradiation in CR1. Thirty-one boys and 21 girls with higher-risk criteria received a stem cell transplant (SCT) in CR1, but no uniform NOPHO indications for SC transplantation or for conditioning therapy existed during the protocol period.¹⁴

Oral 6MP/MTX maintenance therapy. Maintenance therapy was initiated for a total of 1317 patients at treatment week 13 (SR), 32 (IR), or 63 (HR) and scheduled to continue until 2 years (IR and HR) or 2.5 years (SR) after diagnosis. However, for 32 patients the total duration of therapy (and thus also the maintenance therapy phase) was by decision of the treating physician prolonged for more than 12 weeks beyond that of the protocol (median, 15 weeks). For a total of 85 patients, the precise dates for the initiation ($n = 60$) or cessation ($n = 17$) of MTX/6MP maintenance therapy or for both ($n = 8$) were missing from the NOPHO leukemia registry. For those 85 patients, the date for start of MTX/6MP maintenance therapy was estimated from the median number of days from diagnosis for the patients in that risk group for whom the dates were available (SR, 103 days; IR, 245 days; HR, 489 days), whereas the date for cessation of maintenance therapy was set to be 2.0 years (IR- and HR-ALL) or 2.5 years (SR-ALL) from diagnosis. None of those 85 patients developed SMN during CR1. The starting doses of 6MP and MTX were 75 mg/m² per day and 20 mg/m² per week, respectively. During the first year of maintenance therapy, patients with SR- or IR-ALL received alternate pulses at 4-week intervals of (1) VCR (2.0 mg/m² once) and prednisolone (60 mg/m² per day for 1 week) and (2) HD-MTX (5 g/m² every 24 hours with intrathecal MTX and leucovorin rescue) until 5 courses of HD-MTX had been given.¹⁵ Every 8 weeks throughout maintenance therapy, HR patients received reinductions of VCR (1.5 mg/m² once) and prednisolone (40 mg/m² per day for 5 days) with intrathecal MTX. Between 1992 and 1996, 538 patients with SR-, IR-, or HR-ALL were included in the randomized ALL-92 maintenance therapy trial.¹⁶ It explored the prognostic effect of dose adjustments of oral 6MP/MTX maintenance therapy by erythrocyte levels of 6MP/MTX metabolites. TPMT genotype, phenotype, or both were not included in the dose adjustments of that study. As part of that study nearly 30 000 datasets on MTX and 6MP doses as well as parameters for myelotoxicity and hepatotoxicity were registered and used in the present study for comparisons between patients who did or did not develop SMNs. For the purpose of the present study, we also collected data on MTX and 6MP dosing and on leukocyte and absolute neutrophil counts during maintenance therapy for the patients with SMNs who were not included in the randomized study.

LSA₂L₂ maintenance therapy. On the basis of the unsatisfactory results of the NOPHO ALL-86 protocol for patients with higher risk

ALL,^{13,17} NOPHO tested in a nonrandomized study the efficacy of multidrug, cyclic LSA₂L₂ remission maintenance therapy to VHR-patients (all Nordic countries) and to all Finnish patients with HR-ALL. LSA₂L₂ maintenance therapy was scheduled to be given from protocol weeks 48 to 95.¹³ However, antileukemic treatment was discontinued at week 104, even if less than 6 LSA₂L₂ cycles had been due to treatment delays. If a patient were able to complete 6 LSA₂L₂ maintenance therapy cycles before treatment week 104, up to 9 weeks of oral MTX/6MP maintenance therapy was given until week 104. Each LSA₂L₂ block consisted of 4 courses at 2-week intervals with alternating combinations of (1) 6-thioguanine, intravenous cyclophosphamide, and intrathecal MTX, (2) hydroxyurea and daunorubicin (substituted with prednisolone/VCR after the first 4 hydroxyurea/daunorubicin cycles), (3) oral MTX and BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea), and (4) intravenous cytarabine and VCR.^{13,18-20}

TPMT. The TPMT genotype (low-activity polymorphisms G460A and A719G) or erythrocyte TPMT activity or both were available for 609 patients.²¹ For 484 of those patients only the erythrocyte TPMT activity was available, and it was measured 1 to 5 times during maintenance therapy as previously described.²² For patients with more than one TPMT activity measurement, an arithmetic mean TPMT activity was calculated. All TPMT phenotype assays were performed at least 8 weeks after the most recent blood transfusion. The antimode of the TPMT activity distribution was 14 IU/mL. The 62 patients below that level, and for whom no TPMT genotype was available, were classified as TPMT presumed heterozygous ($n = 60$; median TPMT-activity, 10.4 IU/mL; range, 6.2-13.9 IU/mL) or TPMT-deficient ($n = 2$; activity < 1.0 IU/mL). Among the 40 patients for whom both TPMT genotyping and phenotyping were available, all 9 patients who were TPMT heterozygous by genotyping had TPMT activity less than 14.0 IU/mL. However, also 5 (16%) of 31 patients who had a wild-type genotype had TPMT activity less than 14 IU/mL (range, 9.0-13.4 IU/mL), but they were classified by their genotype results as TPMT wild-type. In total, 533 patients were classified as TPMT wild-type, 74 as heterozygous, and 2 as TPMT deficient. In the following analyses the latter 2 subsets were grouped together as TPMT low activity, which included the presumed heterozygous patients (TPMT activity < 14 IU/mL; no genotype available), and the remainder as TPMT wild-type. The TPMT phenotype and genotype were not revealed to the physicians while the patients were on therapy.

Karyotype. Only conventional G-band karyotyping was mandatory. In addition, many patients were explored with high-resolution comparative genomic hybridization, fluorescent in situ hybridization, or reverse transcriptase-polymerase chain reaction for selected translocations [eg, $t(9;22)(q34;q11)$].²³ The karyotypes of the patients were described according to ISCN (International System for Human Cytogenetic Nomenclature) 1995.²⁴ For 664 patients, information on karyotypes was missing, or only normal metaphases were obtained. Of the 950 aberrant karyotypes, 166 had $t(12;21)(p13;q22)$, 362 patients were classified as high-hyperdiploid with a modal chromosome number of 51 to 60 chromosomes, 18 had $t(1;19)(q23;p13)$, 27 had $t(9;22)(q34;q11)$, 11 had MLL rearrangements, 19 had a hypodiploid karyotype (chromosome modal number < 45, including one case with $t(9;22)[BCR/ABL]$), and 348 cases had other chromosomal aberrations.

Statistics

All patients were analyzed according to the treatment they actually received. The duration of 6MP/MTX maintenance therapy was calculated as the number of weeks between the initiation of 6MP/MTX maintenance therapy and performance of a stem cell transplantation in CR1 ($n = 1$), the occurrence of an event, or the end of antileukemic therapy, whichever came first. The average doses of 6MP and MTX were calculated as the total prescribed doses divided by the duration of maintenance therapy. The risk of SMN was calculated from the time of diagnosis, and survival analyses were performed with a basic time scale defined by the date of diagnosis. Patients who died in CR1, received a SCT in CR1, or developed a relapse were censored at the time of these events in the analysis of SMN risk factors. Cox proportional hazard regression analyses were performed with the likelihood ratio test for differences in risk of SMN.^{25,26} Wilcoxon rank sum test was applied to compare the distribution of parameters between

Table 2. Characteristics of second malignant neoplasms in the NOPHO ALL-92 study

Patient ID	Sex	Age*	WBC count*	B/T cells	Karyotype ALL†	Risk‡	TPMT	Interval§	SCT	SMN	t-AML/MDS karyotype†	Post-SMN Tx	Survival after SMN, mo
1	Female	3.3	4	preB	47,XX,-3,add(3)(p11),-7,add(12)(p11),-16,add(16)(p13),+4mar[5]	SR	NA/WT	2.6	No	MDS	46,XX,der(2)(t(2:3)(q32;q13)[8]/46,XX[10]	CTX and SCT	9/D
2	Female	10.0	3	preB	51-53,XX?,inc[2]	SR	NA/NA	2.8	No	AML	46,XX,add(1)(p36)	CTX and SCT	98/A
3	Male	6.1	5	preB	59-60,XXY,-1,-2,-3,-7,-9,add(11)(q29),-13,-15,-16,-18,-19,-20,+21,inc[7]XY[8]	SR	NA/NA	2.9	No	MDS	45,XY,-7[19]/46,XY[5]	CTX and SCT	64/A
4	Male	4.0	5	preB	53-57,X?,inc[6]/46,XY[14]	SR	NA/WT	3.3	No	MDS	43,XY,t(5;7)(q13;q12;q34),-7,-9,-17,-17,-18,+mar[15]	CTX and SCT	8/D
5	Male	3.4	5	preB	54-66,XY,+mar,inc[cp4]/46,XY[20]	SR	11.7/LA	3.3	No	MDS	45,XY,-7[27]	CTX and SCT	12/D
6	Male	4.6	3	preB	ish,t(12;21)(p13;q22)	SR	16.2/WT	3.5	No	AML	45,XY,-7[21]/46,XY[3]	CTX and SCT	20/D
7	Female	9.5	6	preB	46,XX	SR	20.1/NA	3.8	No	MDS	44,XX,+der(5;17)(q10;q10),-5,del(7)(q22q36),dup(11)(q21q25)x4,-17,-22[10]/45,XX,del(4)(q12),+der(5;17)(q10;q10),-5,del(7)(q22q36),-17[10]/46,XX[5]	CTX and SCT	8/D
8	Female	4.4	4	preB	58,XX,-X,-1,-2,-3,-4,-5,-7,-8,-9,-12,-13,-16,-19,-20,-22,+mar,inc	SR	12.1/LA	3.9	No	MDS	45,XX,add(13)(31)	CTX and SCT	13/D
9	Female	2.5	4	preB	54,XX,+X,+5,+6,+10,+14,+17,inc[11]	SR	NA/NA	4.5	No	AML	46,XX,? del(7)(q12)[1]/45-46,XX,-5,?del(7)(q),+mar[2]/46,XX[2]	CTX and SCT	65/A
10	Female	4.3	3	preB	53,XX,+X,+4,+21,inc[13]	SR	NA/WT	4.6	No	AML	46,XX,add(1)(p36),der(5)(5:6)(?;p23)[25]	CTX	42/A
11	Female	2.9	6	preB	46,XX	SR	20.0/NA	5.3	No	AML	46,XX,add(1)(p),-7,+mar[24]	CTX and SCT	10/D
12	Female	2.3	4	preB	46,XX[?],por.(12;21)(p13;q22)	SR	15.6/WT	6.6	No	MDS	45-47,XX,add(3)(p24),der(4)t(4;?) (q28;?), der(5)t(5;?) (q32;?),-6,-9,-10,+2mar,inc[5]/46,XX[6]	CTX and SCT	18/A
13	Female	1.5	11	preB	46,XX	IR	10.7/WT	1.9	No	AML	45,XX,-7,der(8)(8;10)(q24;q24)[16]/46,XX[10]	CTX	6/D
14	Female	13.9	40	preB	58-70,XX,inc	IR	NA/WT	2.2	No	PNET		Surgery and RTx	16/D
15	Female	12.9	3	preB	46,XX	IR	NA/NA	7.5	No	MDS	46,XX,-21,+mar[12]/45,XX,-7,-21,+mar[cp3]/46,XX[5]	CTX and SCT	38/A
16	Male	4.7	38	preB	NA	IR	10.6/NA	11.6	No	Oral c		Surgery	5/D
17	Male	12.0	37	T	46,XY	HR	10.5/LA	2.0	No	AML	46,XY,del(11)(q23)[10]	CTX	6/D
18	Male	5.8	4	preB	ish,t(12;21)(p13;q22)	HR	NA/NA	5.5	Yes	AML¶	48,+2mar,inc(1)/46,XY;nuc ish 12p13(TELx2),21q22(AML1x4-8)	CTX and SCT	31/A
19	Male	10.7	116	preB	46,XY,t(9;22)(q34;q11)[27]/46,XY[3]	VHR	NA/NA	0.8	Yes	PTLD		CTX	0/D
20	Male	14.2	106	preB	51-58,+X?,+47,+21,+21,ish,der(22)t(9;22;16)(q34;q11;q22)[14]	VHR	NA/NA	3.3	Yes	Thyroid		Surgery and RTx	51/A

Patients are sorted by risk group and interval between diagnosis of ALL and SMN.
WBC indicates white blood cell; ALL, acute lymphoblastic leukemia; TPMT, thiopurine methyltransferase activity in red blood cells (in IU/mL)/genotype; SCT, stem cell transplantation; SMN, second malignant neoplasm; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; Tx, treatment; B and T, B- (pre-B) and T-lineage ALL; A, alive; CTx, chemotherapy; D, dead; NA, not available; Oral c, oral carcinoma; PNET, primitive neuroectodermal tumor in central nervous system; PTLD, post-transplantation lymphoproliferative disease (not Epstein-Barr virus-related); RTx, radiotherapy; t-, therapy-related; thyroid, thyroid carcinoma; LA, heterozygous with 1 low activity allele; and WT, wild-type.
*Age (years) and WBC count (cells × 10⁹/L) at diagnosis of ALL.
†Karyotype by G-band karyotyping, fluorescence in situ hybridization, or both.
‡SR/IR/HR/VHR indicates standard/intermediate/high/very high risk ALL.
§Interval in years between diagnosis of ALL and SMNs.
¶AML for the SMN.
||AML in donor cells only (after SCT).

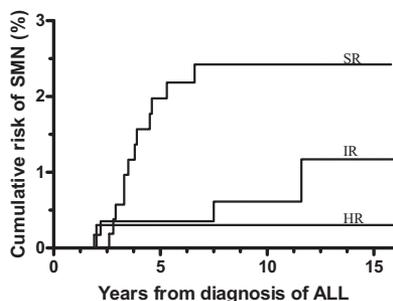


Figure 2. Cumulative risk of a second malignant neoplasm (SMN) by risk group for patients who did not receive a transplant in first remission. Standard risk (SR) was $2.4\% \pm 0.7\%$, intermediate risk (IR) was $1.2\% \pm 0.7\%$, and higher risk (HR) was $0.3\% \pm 0.3\%$ ($P = .007$). ALL indicates acute lymphoblastic leukemia.

subgroups.²⁷ The Kaplan-Meier method was applied to estimate remission duration and to generate survival curves.²⁸ Subgroups were compared with the log-rank test.²⁹ Two-sided P values less than .05 were regarded as significant.

Results

The median follow-up time for the 1225 patients who remain in first remission is 10.4 years (50% range, 8.0-12.6 years). At the end of the study, 34 patients had died in CR1, 335 patients had developed a relapse of ALL 0.2 to 12.0 years from diagnosis (median, 2.6 years), and 20 patients had developed an SMN (Figure 1). The overall projected 12-year event-free survival and overall survival were $75\% (\pm 1\%)$ and $86\% (\pm 1\%)$, respectively.

The 20 SMNs, including 3 that occurred after SC transplantation, were diagnosed at a median of 3.4 years (range, 0.8-11.6 years) from the initial diagnosis of ALL (Table 2). All SMNs in patients who did not receive a transplant occurred after cessation of maintenance therapy. None of the patients with Down syndrome developed an SMN. Sixteen of the 20 SMNs were AML ($n = 8$) or MDS ($n = 8$), all but 2 of which occurred within 5.5 years from diagnosis (median, 3.7 years). One of these AMLs occurred after SC transplantation. The last 4 patients included a primitive neuroectodermal CNS tumor, an oral carcinoma, and 2 post-SC transplantation cancers (a posttransplantation, lymphoproliferative disease and a papillary thyroid cancer). The 12-year cumulative risk of developing an SMN ($pSMN_{12y}$) was 1.6% plus or minus 0.4% . Thirteen of the 16 patients with t-AML/MDS received a SCT. Twelve of the 20 patients died within 21 months from diagnosis of their SMN with a median time to death of 8.8 months and a projected survival fraction of 0.39 plus or minus 0.11 (Table 2).

The projected risk of SMN among the patients who did not receive a transplant was significantly related to the risk group and was $2.4\% (\pm 0.7\%)$ for SR-ALL, $1.2\% (\pm 0.7\%)$ for IR-ALL, and $0.3\% (\pm 0.3\%)$ for higher risk ALL patients ($P = .007$; Figure 2).

Of the 16 SMNs (15 t-AML/MDS) that occurred among the SR/IR patients, 10 patients had an aberrant karyotype at the time of ALL diagnosis of which 8 were high-hyperdiploid compared with 301 high-hyperdiploid cases among the 684 SR/IR patients with an aberrant karyotype who did not develop an SMN ($P = .03$). This reaches borderline significance if all successful karyotypes are included as the denominator ($P = .07$). Among all 562 SR-ALL patients, the 161 patients with a high-hyperdiploid karyotype had a $4.8\% (\pm 1.8\%)$ risk of developing an SMN compared with a $1.5\% (\pm 0.6\%)$ risk for the remaining 401 SR patients ($P = .02$). No other subsets of genetic aberrations were significantly related to the risk of developing an SMN.

In all 16 cases of t-AML/MDS a karyotype was available and showed chromosomal aberrations in all, including one 11q23-aberration, monosomy 7 ($n = 7$), 7q deletion ($n = 2$), or chromosome 5 deletions or translocations ($n = 5$; Table 2). Cytogenetic aberrations involving chromosomes 5 or 7 occurred in 11 of 15 patients with t-AML/MDS who did not receive a transplant. The 9 -7/7q- t-AML/MDS cases occurred among patients with high-hyperdiploidy ($n = 4$), $t(12;21)(p13;q22)$ ($n = 1$), or a normal/missing ($n = 4$) karyotype at diagnosis of ALL. The projected survival of the 9 -7/7q- t-AML/MDS did not differ significantly from that of the remaining 11 SMNs (Table 2).

The cumulative risk of developing an SMN was $1.7\% (\pm 0.4\%)$ for the 1316 patients who were in CR1 when they initiated MTX/6MP maintenance therapy compared with 0% for the 169 patients in CR1 at the start of LSA₂L₂ maintenance therapy.

To analyze the effect of MTX/6MP maintenance therapy on the risk of SMN, the 1562 patients who did not receive a SCT in CR1 were grouped according to whether they received MTX/6MP maintenance therapy and its duration in weeks. In backward, multivariate Cox regression analysis of the SR/IR/HR-ALL patients who had received MTX/6MP maintenance therapy only the duration of oral MTX/6MP maintenance therapy in weeks (regression coefficient, 0.02; $P = .04$) was related to an increased risk of an SMN, whereas neither sex, age, and white blood cell count at diagnosis came out statistically significant. If the analysis included the presence of a high-hyperdiploid karyotype, only the duration of MTX/6MP maintenance therapy (regression coefficient, 0.02; $P = .05$) and presence of high-hyperdiploidy ($P = .07$) were related to an increased risk of an SMN (overall P value of the Cox model, .02). Finally, inclusion of the VHR patients, most of whom only received LSA₂L₂ maintenance therapy, yielded the same coefficients for the duration of MTX/6MP maintenance therapy (regression coefficient, 0.02; $P = .02$) and presence of high-hyperdiploidy ($P = .07$; overall P value of the Cox model, .003). Within the individual SR-, IR-, and HR-ALL risk groups, the patients who did or did not develop SMN did not differ significantly in their actual duration of MTX/6MP maintenance therapy ($P > .2$ in all comparisons).

With the limitation that data on MTX and 6MP doses, erythrocyte metabolite levels, or blood counts were only available for the 545 patients who participated in the randomized NOPHO ALL-92 maintenance therapy study or developed an SMN (Figure 1), the patients who developed an SMN did not differ significantly from the remaining population with respect to their doses of 6MP (median, 62.4; $n = 16$ vs 59.4 mg/m²; $n = 526$; $P = .18$) or MTX (median, 16.6; $n = 16$ vs 15.3 mg/m²; $n = 526$; $P = .21$), their erythrocyte MTX levels (median, 4.9; $n = 8$ vs 5.6 nmol/mmol hemoglobin [Hb]; $n = 514$; $P = .17$), their erythrocyte 6TGN levels (median, 297 vs 170 nmol/mmol Hb; $P = .11$), or their average leukocyte (median: 3.4; $n = 17$ vs $3.3 \times 10^9/L$; $n = 527$; $P = .35$) or absolute neutrophil counts (median, 2.1; $n = 17$ vs $2.0 \times 10^9/L$; $n = 529$; $P = .91$). In contrast, the patients who developed an SMN had significantly lower TPMT activity than did the remaining patients (median, 12.1 vs 18.1 IU/mL; $P = .02$; Figure 3). The patients who developed an SMN received 10% to 15% higher 6MP doses than those that did not develop an SMN, and this was the case both among patients classified as TPMT low activity (median, 55.5; $n = 3$ vs 50.3 mg/m²; $n = 57$; $P = .31$) and those classified as TPMT wild-type (median, 69.7; $n = 9$ vs 60.4 mg/m²; $n = 418$; $P = .03$). The patients with lower TPMT activity tended to be diagnosed with t-AML/MDS earlier than patients with higher TPMT activity, but this tendency did not reach

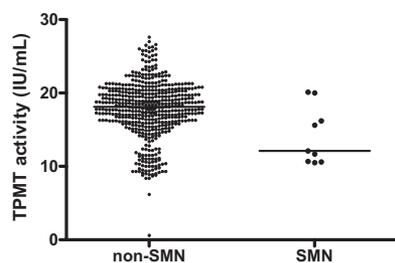


Figure 3. Red blood cell thiopurine methyltransferase activity (TPMT) for patients who developed a second malignant neoplasm compared with patients who did not. Nine patients developed a SMN (right) compared with 515 patients who did not (left). Each dot represents 1 patient (median, 12.1 vs 18.1 IU/mL; $P = .02$).

statistical significance in Spearman correlation analysis ($r_s = 0.67$, $P = .07$). Four of the 76 patients classified as TPMT low activity developed an SMN ($pSMN_{12y} = 6\% \pm 3\%$), compared with 9 of 533 classified as TPMT wild-type patients ($pSMN_{12y} = 2\% \pm 1\%$; $P = .06$).

The overall risk of SMN in the randomized NOPHO ALL-92 maintenance therapy study did not differ between the 2 treatment arms (4 cases among 269 patients in each arm) and did not differ from that of 1024 patients who did not receive a transplant who did not participate in the study ($P = .9$).

Discussion

Today, the most effective treatment protocols for children with ALL result in cure rates of 80% to 85%.^{30,31} In this perspective the life-threatening complications of antileukemic therapy such as therapy-related SMN increase their relative effect on the overall survival. Although the risk of SMN within the first 15 years from diagnosis is only a few percent in most protocols,^{1,3-5,13,32,33} subsets of patients with significantly higher risk have been identified. These subsets include patients exposed to topoisomerase II inhibitors or alkylating agents, in which the genotoxicity and relation to malignant transformation is well established.^{5,34} Furthermore, after CNS irradiation the cumulative risk of head and neck secondary neoplasms and CNS tumors, including meningiomas, may be well above 10% at 25 to 30 years from diagnosis of ALL.^{1,35} Finally, several drugs have been suggested to modify the risk of SMNs by interference with DNA repair or by promoting the growth of a malignant clone. Such agents include L-asparaginase,³⁶ granulocyte colony-stimulating factor,³⁷ and thiopurines.^{7,8,38}

The present population-based, retrospective study indicates that maintenance therapy, and especially 6MP pharmacotherapy, influenced the development of SMNs. This conclusion is based on several lines of evidence. First, the strongest indication that 6MP influenced the risk of the SMN is the known DNA-damaging effect of 6MP³⁹⁻⁴¹ and the correlation of the risk of t-AML/MDS to (1) longer duration of 6MP/MTX maintenance therapy, (2) low TPMT activity, (3) higher doses of 6MP for TPMT wild-type patients, and (4) higher 6TGN levels (although nonsignificant). During oral 6MP therapy 1:10³ to 1:10⁴ DNA nucleotides in leukocytes have been shown to be substituted with 6TGN (DNA-6TGN),^{42,43} but at present it is unknown whether the risk of t-AML/MDS is related to the individual leukocyte DNA-6TGN levels. Second, none of the patients on NOPHO ALL-92 received epipodophyllotoxins, which are known to be leukemogenic, at least in part because of inhibition of topoisomerase II.^{34,44} Third, although all patients received anthracyclines during induction

therapy and delayed intensification, the total dose for SR-ALL patients was only 120 mg/m². However, malignant clones may arise a few months after anthracyclines have been administered, and the subsequent therapy may then have modified the risk of whether it develops into overt t-AML/MDS,⁴⁵ and anthracycline therapy during induction could thus have been a contributing factor for the development of t-AML/MDS in the present cohort. Fourth, only the patients with IR-ALL, HR-ALL, and VHR-ALL received an alkylating agent (3.0-6.6 g/m² of cyclophosphamide), and none of the SMNs occurred among the CNS-irradiated patients who did not receive a transplant. Finally, oral low-dose MTX has rarely been reported to induce secondary MDS/AML.⁴⁶ However, HD-MTX could have been a contributing factor through its inhibition of purine de novo synthesis and enhancement of DNA-6TGN incorporation during the concurrently administered 6MP.^{47,48} Accordingly, patients with low TPMT activity are more prone to develop severe myelotoxicity after HD-MTX with concurrently administered 6MP.⁴⁹

As a result of a common genetic polymorphism, approximately 90% of whites are homozygous for high TPMT activity (TPMT wild-type), 5% to 10% are heterozygous and express intermediate TPMT activity, and 1:300 are TPMT deficient.¹¹ Levels of TPMT activity in red blood cells, lymphocytes, and leukemic blasts are generally closely correlated, although *TPMT* gene copy number changes may modify the TPMT phenotype of the leukemic cells.⁵⁰

TPMT methylates 6MP and other 6MP metabolites such as 6-thioinosine 5'-monophosphate, producing 6-methyl-mercaptopurine and 6-methyl-thioinosine 5'-monophosphate (MeTIMP), respectively.¹¹ MeTIMP is a strong inhibitor of purine de novo synthesis,¹¹ and MeTIMP levels increase with higher doses of 6MP,¹² which may explain the positive correlation between the dose of 6MP and the risk of SMN among the TPMT wild-type patients in the present study, because inhibition of purine de novo synthesis could have enhanced DNA-6TGN incorporation.

Reduced TPMT activity increases the availability of 6MP for 6TGN formation, resulting in higher E-6TGN, increased myelotoxicity, and a higher cure rate.^{16,21,51} Because (1) only the 2 most common low-activity polymorphisms were analyzed in the present cohort, (2) the concordance between TPMT genotype and phenotype is incomplete, and (3) erythrocyte TPMT activity may fluctuate during therapy because of variation in the life span of red blood cell,⁵² it remains to be explored whether TPMT genotyping or phenotyping is superior in prediction of the risk of SMNs. Since 2001, determination of TPMT genotype/phenotype has been mandatory in NOPHO protocols, and during the initial maintenance therapy 6MP dose is reduced to 50 mg/m² for TPMT heterozygous patients. With longer follow-up it will be explored whether this approach has reduced the risk of SMNs for such patients without increasing their low relapse rate.²¹

In support of the present findings, St Jude Children's Research Hospital has previously reported that the interval between the diagnosis of ALL and of t-AML/MDS was correlated with TPMT activity.³⁸ In contrast, the German Berlin-Frankfurt-Münster (BFM) study group failed to show a significant relation between low-activity TPMT genotypes and the risk of SMNs.⁵³ However, (1) only genotyping and not phenotyping was used in the BFM study; (2) the backbone maintenance therapy dose of 6MP was 75 mg/m² in the NOPHO protocol compared with only 50 mg/m² in the BFM protocol; (3) the total duration of therapy for the SR-ALL patients was 27 months in NOPHO ALL-92, whereas maintenance therapy lasts only approximately 18 months for SR-patients in BFM protocols⁵⁴; and (4) the BFM protocol only

give 6MP 25 mg/m² concurrent with HD-MTX (so-called protocol M), whereas the NOPHO ALL-92 protocol prescribed HD-MTX (5 g/m²) 5 times together with concurrent 6MP at 75 mg/m² during maintenance therapy, which could have increased 6MP-induced DNA damage.^{49,55}

Although the pattern of second cancers may change with longer follow-up, the high relative frequency of t-AML/MDS and their genetic aberrations in the present study is different from many previous reports, in which solid tumors were relatively more common and t-AML/MDS frequently harbored 11q23-aberrations because of more extensive exposure to topoisomerase II inhibitors such as epipodophyllotoxins and anthracyclines.^{34,44,54} Furthermore, t-AML/MDS with deletions of all or part of chromosomes 5 and 7 have previously been linked to exposures to alkylating agents or radiation therapy.⁵ Note that only one 11q23-rearranged case was found in the present cohort [a del(11q23)], whereas 14 cases had chromosome 5 or 7 aberrations. A linkage between high-hyperdiploid ALL and SMN, including t-AML/MDS with -7/7q-, has not previously been reported, but thiopurine therapy has been linked to t-AML/MDS with monosomy 7 in patients with benign disorders.^{41,56} It is uncertain to what extent the ALL cases with missing or normal karyotypes in reality had a high-hyperdiploid clone, but it is well known that conventional chromosome analysis may fail to identify high-hyperdiploidy because of poor in vitro growth of leukemic cells in this ALL subset.²³ Because both high-hyperdiploidy and monosomy 7 represent mitotic nondisjunction, the linkage could represent an individual propensity for such mitotic errors similar to that identified in mothers of children with Down syndrome.⁵⁷

Partly explained by pharmacogenetic polymorphisms, children with ALL vary significantly in their drug disposition and treatment response,⁵⁸ and some, although not all, studies have linked excessive risks of the development of SMN to specific predisposing genetic polymorphisms such as low-activity glutathione S-transferase, methylenetetrahydrofolate reductase, NAD(P)H:quinone oxidoreductase 1, or TPMT polymorphisms.^{7,8,38,59-64} However, little is known about how to modify therapy to counteract such genetic predisposition. If the linkage of t-AML/MDS with high-hyperdiploidy, TPMT low activity, and 6MP treatment intensity is confirmed by others, studies are needed to explore whether individualized 6MP dose adjustments can reduce their risk of this sequelae to antileukemic therapy without increasing their rate of relapse.

References

- Hijji N, Hudson MM, Lensing S, et al. Cumulative incidence of secondary neoplasms as a first event after childhood acute lymphoblastic leukemia. *JAMA*. 2007;297:1207-1215.
- Garwicz S, Anderson H, Olsen JH, et al. Second malignant neoplasms after cancer in childhood and adolescence: a population-based case-control study in the 5 Nordic countries. *Int J Cancer*. 2000;88:672-678.
- Bhatia S, Sather HN, Pabustan OB, et al. Low incidence of second neoplasms among children diagnosed with acute lymphoblastic leukemia after 1983. *Blood*. 2002;99:4257-4264.
- Loning L, Zimmermann M, Reiter A, et al. Secondary neoplasms subsequent to Berlin-Frankfurt-Munster therapy of acute lymphoblastic leukemia in childhood: significantly lower risk without cranial radiotherapy. *Blood*. 2000;95:2770-2775.
- Ng A, Taylor GM, Eden OB. Treatment-related leukaemia: a clinical and scientific challenge. *Cancer Treat Rev*. 2000;26:377-391.
- Nygaard R, Garwicz S, Haldorsen T, et al. Second malignant neoplasms in patients treated for childhood leukemia. A population-based cohort study from the Nordic countries. The Nordic Society of Pediatric Oncology and Hematology (NOPHO). *Acta Paediatr Scand*. 1991;80:1220-1228.
- Thomsen JB, Schroder H, Kristinsson J, et al. Possible carcinogenic effect of 6-mercaptopurine on bone marrow stem cells: relation to thiopurine metabolism. *Cancer*. 1999;86:1080-1086.
- Relling MV, Rubnitz JE, Rivera GK, et al. High incidence of secondary brain tumours after radiotherapy and antimetabolites. *Lancet*. 1999;354:34-39.
- Van Scoik KG, Johnson CA, Porter WR. The pharmacology and metabolism of the thiopurine drugs 6-mercaptopurine and azathioprine. *Drug Metab Rev*. 1985;16:157-174.
- Lennard L. The clinical pharmacology of 6-mercaptopurine. *Eur J Clin Pharmacol*. 1992;43:329-339.
- Wang L, Weinshilboum R. Thiopurine S-methyltransferase pharmacogenetics: insights, challenges and future directions. *Oncogene*. 2006;25:1629-1638.
- Erb N, Harms DO, Janka-Schaub G. Pharmacokinetics and metabolism of thiopurines in children with acute lymphoblastic leukemia receiving 6-thioguanine versus 6-mercaptopurine. *Cancer Chemother Pharmacol*. 1998;42:266-272.
- Gustafsson G, Schmiegelow K, Forestier E, et al. Improving outcome through two decades in childhood ALL in the Nordic countries: the impact of high-dose methotrexate in the reduction of CNS irradiation. *Nordic Society of Pediatric Haematology and Oncology (NOPHO)*. *Leukemia*. 2000;14:2267-2275.
- Saaren-Pihkala UM, Heilmann C, Winiarski J, et al. Pathways through relapses and deaths of children with acute lymphoblastic leukemia: role of allogeneic stem-cell transplantation in Nordic data. *J Clin Oncol*. 2006;24:5750-5762.
- Skarby TV, Anderson H, Heldrup J, et al. High leucovorin doses during high-dose methotrexate treatment may reduce the cure rate in childhood

Acknowledgments

We thank all the Nordic pediatric oncology centers that have supported this study with registration of patient data.

This was supported by The Danish Childhood Cancer Foundation; The University Hospital Rigshospitalet; The Children's Cancer Foundation of Sweden (grants 53/91, 62/94, 72/96, 98/59, 04/002); The Danish Cancer Society (grants 91-048, 92-017, 93-017, 95-100-28); The Lundbeck Foundation (grant 38/99); NovoNordisk Foundation; Home Secretary Research Grant for Individualised Therapy; Danish Research Council for Health and Disease; The Nordic Cancer Union (grants 56-9257, 56-100-03-9102); The Otto Christensen Foundation; and the United States National Institutes of Health (NIH; grants R01-GM28157, U01 GM61388).

K.S. holds the Danish Childhood Cancer Foundation Research Professorship in Pediatric Oncology.

Authorship

Contribution: K.S. designed the study and wrote the paper; R.W. performed most of the TPMT analyses; K.S. and J.N. performed the rest of the pharmacologic analyses; M.K.A. performed karyotyping; E.F. (together with the Nordic Society for Paediatric Haematology and Oncology cytogenetic group) scrutinized the karyotypes of all patients; H.H. was responsible for verifying t-AML/MDS cases; H.H., I.A.-M., M.B., J.K., M.H., K.V., and R.N. were responsible for providing clinical data for the patients; K.S. and A.L.S. performed the statistical analyses; and all authors commented and approved the final manuscript.

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

A complete list of the members of the Nordic Society for Paediatric Haematology and Oncology study group appears in the Appendix (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Correspondence: Kjeld Schmiegelow, The Pediatric Clinics, Juliane Marie Center 5704, University Hospital Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark; e-mail: kschmiegelow@rh.dk.

- acute lymphoblastic leukemia. *Leukemia*. 2006; 20:1955-1962.
16. Schmiegelow K, Bjork O, Glomstein A, et al. Intensification of mercaptopurine/methotrexate maintenance chemotherapy may increase the risk of relapse for some children with acute lymphoblastic leukemia. *J Clin Oncol*. 2003;21:1332-1339.
 17. Gustafsson G, Kreuger A, Clausen N, et al. Intensified treatment of acute childhood lymphoblastic leukaemia has improved prognosis, especially in non-high-risk patients: the Nordic experience of 2648 patients diagnosed between 1981 and 1996. *Nordic Society of Paediatric Haematology and Oncology (NOPHO). Acta Paediatr*. 1998;87: 1151-1161.
 18. Shuster JJ, Falletta JM, Pullen DJ, et al. Prognostic factors in childhood T-cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. *Blood*. 1990;75:166-173.
 19. Steinherz PG, Gaynon PS, Breneman JC, et al. Treatment of patients with acute lymphoblastic leukemia with bulky extramedullary disease and T-cell phenotype or other poor prognostic features: randomized controlled trial from the Children's Cancer Group. *Cancer*. 1998;82:600-612.
 20. Saarinen-Pihkala UM, Lanning M, Perkkio M, et al. Granulocyte-macrophage colony-stimulating factor support in therapy of high-risk acute lymphoblastic leukemia in children. *Med Pediatr Oncol*. 2000;34:319-327.
 21. Schmiegelow K, Forestier E, Kristinsson J, et al. Thiopurine methyltransferase activity is related to the risk of relapse of childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. *Leukemia*. 2009;23:557-564.
 22. Weinshilboum RM, Raymond FA, Pazmino PA. Human erythrocyte thiopurine methyltransferase: radiochemical microassay and biochemical properties. *Clin Chim Acta*. 1978;85:323-333.
 23. Kristensen TD, Wesenberg F, Jonsson OG, et al. High-resolution comparative genomic hybridisation yields a high detection rate of chromosomal aberrations in childhood acute lymphoblastic leukaemia. *Eur J Haematol*. 2003;70:363-372.
 24. Mitelman F. An international system for human cytogenetic nomenclature. Basel, Switzerland: S Karger; 1995.
 25. Cox DR. Regression models and life-tables (with discussion). *J R Stat Soc (B)*. 1972;34:187-220.
 26. Andersen PK, Borgan O, Gill RD, Keiding N. Statistical models based on counting processes. New York, NY: Springer-Verlag; 1993.
 27. Siegel S, Castellan NJ. Nonparametric statistics for the behavioral sciences. Singapore: McGraw-Hill; 1988.
 28. Kaplan EJ, Meier P. Non-parametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
 29. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother*. 1966;50:163-170.
 30. Schrappe M, Camitta B, Pui CH, et al. Long-term results of large prospective trials in childhood acute lymphoblastic leukemia. *Leukemia*. 2000; 14:2193-2194.
 31. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med*. 2006;354:166-178.
 32. Pui CH, Boyett JM, Rivera GK et al. Long-term results of total therapy studies 11, 12 and 13A for childhood acute lymphoblastic leukemia at St Jude Children's Research Hospital. *Leukemia*. 2000;14:2286-2294.
 33. Maule M, Scelo G, Pastore G, et al. Risk of second malignant neoplasms after childhood leukemia and lymphoma: an international study. *J Natl Cancer Inst*. 2007;99:790-800.
 34. Pedersen-Bjergaard J, Christiansen DH, Andersen MK, Skovby F. Causality of myelodysplasia and acute myeloid leukemia and their genetic abnormalities. *Leukemia*. 2002;16:2177-2184.
 35. Goshen Y, Stark B, Kornreich L, et al. High incidence of meningioma in cranial irradiated survivors of childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2007;49:294-297.
 36. Pui CH, Relling MV, Behm FG, et al. L-asparaginase may potentiate the leukemogenic effect of the epipodophyllotoxins. *Leukemia*. 1995;9:1680-1684.
 37. Relling MV, Boyett JM, Blanco JG, et al. Granulocyte colony-stimulating factor and the risk of secondary myeloid malignancy after etoposide treatment. *Blood*. 2003;101:3862-3867.
 38. Relling MV, Yanishevski Y, Nemecek J, et al. Etoposide and antimetabolite pharmacology in patients who develop secondary acute myeloid leukemia. *Leukemia*. 1998;12:346-352.
 39. Waters TR, Swann PF. Cytotoxic mechanism of 6-thioguanine: hMutSalpha, the human mismatch binding heterodimer, binds to DNA containing S6-methylthioguanine. *Biochemistry*. 1997;36:2501-2506.
 40. Swann PF, Waters TR, Moulton DC, et al. Role of postreplicative DNA mismatch repair in the cytotoxic action of thioguanine. *Science*. 1996;273: 1109-1111.
 41. Karran P, Attard N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. *Nat Rev Cancer*. 2008;8:24-36.
 42. Olesen KM, Honore S, Sidenius U, Schmiegelow K. Determination of DNA 6-thioguanine nucleotide levels by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B*. 2008;864:149-155.
 43. Warren DJ, Andersen A, Sordal L. Quantitation of 6-thioguanine residues in peripheral blood leukocyte DNA obtained from patients receiving 6-mercaptopurine-based maintenance therapy. *Cancer Res*. 1995;55:1670-1674.
 44. Pui CH, Ribeiro RC, Hancock ML, et al. Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphoblastic leukemia. *N Engl J Med*. 1991;325:1682-1687.
 45. Blanco JG, Dervieux T, Edick MJ, et al. Molecular emergence of acute myeloid leukemia during treatment for acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A*. 2001;98:10338-10343.
 46. Rosenthal NS, Farhi DC. Myelodysplastic syndromes and acute myeloid leukemia in connective tissue disease after single-agent chemotherapy. *Am J Clin Pathol*. 1996;106:676-679.
 47. Bokkerink JP, De Abreu RA, van Laarhoven JP, et al. Increased availability of phosphoribosyl pyrophosphate as the basis for enhanced 6-mercaptopurine incorporation by methotrexate, in cultured human lymphoblasts. *Adv Exp Med Biol*. 1986;195(Pt B):135-139.
 48. Schmiegelow K, Bretton-Meyer U. 6-mercaptopurine dosage and pharmacokinetics influence the degree of bone-marrow toxicity following high-dose methotrexate in children with acute lymphoblastic leukemia. *Leukemia*. 2001;15:74-79.
 49. Andersen JB, Szumlanski C, Weinshilboum RM, Schmiegelow K. Pharmacokinetics, dose adjustments, and 6-mercaptopurine/methotrexate drug interactions in two patients with thiopurine methyltransferase deficiency. *Acta Paediatr*. 1998;87: 108-111.
 50. Cheng Q, Yang W, Raimondi SC, et al. Karyotypic abnormalities create discordance of germline genotype and cancer cell phenotypes. *Nat Genet*. 2005;37:878-882.
 51. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J Natl Cancer Inst*. 1999;91:2001-2008.
 52. Lennard L, Chew TS, Lilleyman JS. Human thiopurine methyltransferase activity varies with red blood cell age. *Br J Clin Pharmacol*. 2001;52:539-546.
 53. Stanulla M, Schaeffeler E, Moericke A, et al. Thiopurine methyltransferase genotype is not a risk factor for secondary malignant neoplasias after treatment for childhood acute lymphoblastic leukemia on Berlin-Frankfurt-Münster protocols [abstract]. *Blood*. 2006;108:48a-49a. Abstract 150.
 54. Moricke A, Reiter A, Zimmermann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. *Blood*. 2008;111:4477-4489.
 55. Nygaard U, Schmiegelow K. Dose reduction of coadministered 6-mercaptopurine decreases myelotoxicity following high-dose methotrexate in childhood leukemia. *Leukemia*. 2003;17:1344-1348.
 56. Yenson PR, Forrest D, Schmiegelow K, Dalal BI. Azathioprine-associated acute myeloid leukemia in a patient with Crohn's disease and thiopurine S-methyltransferase deficiency. *Am J Hematol*. 2008;83:80-83.
 57. Migliore L, Boni G, Bernardini R, et al. Susceptibility to chromosome malsegregation in lymphocytes of women who had a Down syndrome child in young age. *Neurobiol Aging*. 2006;27:710-716.
 58. Davidsen ML, Dalhoff K, Schmiegelow K. Pharmacogenetics influence treatment efficacy in childhood acute lymphoblastic leukemia. *J Pediatr Hematol Oncol*. 2008;30:831-849.
 59. Stanulla M, Dnybil C, Bartels DB, et al. The NQO1 C609T polymorphism is associated with risk of secondary malignant neoplasms after treatment for childhood acute lymphoblastic leukemia: a matched-pair analysis from the ALL-BFM study group. *Haematologica*. 2007;92:1581-1582.
 60. Blanco JG, Edick MJ, Hancock ML, et al. Genetic polymorphisms in CYP3A5, CYP3A4 and NQO1 in children who developed therapy-related myeloid malignancies. *Pharmacogenetics*. 2002;12: 605-611.
 61. Jazbec J, Aplenc R, Dolzan V, Debeljak M, Jereb B. GST polymorphisms and occurrence of second neoplasms after treatment of childhood leukemia. *Leukemia*. 2003;17:2540-2542.
 62. Hartford C, Yang W, Cheng C, et al. Genome scan implicates adhesion biological pathways in secondary leukemia. *Leukemia*. 2007;21:2128-2136.
 63. Woo MH, Shuster JJ, Chen C, et al. Glutathione S-transferase genotypes in children who develop treatment-related acute myeloid malignancies. *Leukemia*. 2000;14:232-237.
 64. Stanulla M, Loning L, Welte K, Schrappe M. Secondary brain tumours in children with ALL. *Lancet*. 1999;354:1126-1127.