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EDUCATIONAL REPORT

Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia

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Analysis of 2668 children with acute lymphoblastic leukemia (ALL) treated in two successive Nordic clinical trials (Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL-92 and ALL-2000) showed that 75% of all patients are cured by first-line therapy, and 83% are long-term survivors. Improvements in systemic and intrathecal chemotherapy have reduced the use of central nervous system (CNS) irradiation to <10% of the patients and provided a 5-year risk of isolated CNS relapse of 2.6%. Improved risk stratification and chemotherapy have eliminated the previous independent prognostic significance of gender, CNS leukemia and translocation t(1;19)(q23;p13), whereas the post-induction level of minimal residual disease (MRD) has emerged as a new risk grouping feature. Infant leukemia, high leukocyte count, T-lineage immunophenotype, translocation t(4;11)(q21;q23) and hypodiploidy persist to be associated with lower cure rates. To reduce the overall toxicity of the treatment, including the risk of therapy-related second malignant neoplasms, the current NOPHO ALL-2008 protocol does not include CNS irradiation in first remission, the dose of 6-mercaptopurine is reduced for patients with low thiopurine methyltransferase activity, and the protocol restricts the use of hematopoietic stem cell transplantation in first remission to patients without morphological remission after induction therapy or with high levels of MRD after 3 months of therapy.
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Introduction

Better understanding of the disease biology and the pharmacology of anticancer agents as well as performance of randomized clinical trials have dramatically improved the cure rates of childhood acute lymphoblastic leukemia (ALL).[1,2] In 1981, the Nordic Society of Paediatric Haematology and Oncology (NOPHO) started a common registration of all children below 15 years of age at diagnosis of ALL in the five Nordic countries (Denmark, Finland, Iceland, Norway and Sweden).[3] During the following decade the therapy became harmonized, and since January 1992 Nordic children 1.0–14.9 years of age have been treated according to common protocols. The details and treatment results of two previous non-uniform protocol periods 1981–1985 and 1986–1991 were reported in the December 2000 issue of *Leukemia* together with those of 11 other study groups,[4] and their overall results are presented in Table 1.

The goals of the first common NOPHO protocol (NOPHO ALL-92) were to replace cranial irradiation with intravenous high-dose chemotherapy for all but the highest risk patients, to examine in detail the significance of oral methotrexate (MTX)/6-mercaptopurine (6MP) maintenance therapy, and to centrally and prospectively scrutinize the karyotypes of all patients. The study showed the feasibility of reduced use of cranial irradiation,[4] emphasized that host pharmacogenetics influence the cure rate,[5] confirmed the importance of myelosuppression during maintenance therapy,[6] showed that MTX/6MP maintenance therapy is important for high-risk (HR) ALL,[7] and allowed mapping of the epidemiological and clinical characteristics of several cytogenetically defined subsets of ALL.[8–14] The goals of the subsequent common NOPHO ALL-2000 protocol were to examine the efficacy of Vincristine (VCR)/Dexamethasone reinductions during maintenance therapy and to examine the feasibility of non-centralized minimal residual disease (MRD) monitoring by flow cytometry and PCR.[15–19] The ALL-92 and ALL-2000 studies have paved the way to total elimination of prophylactic cranial irradiation in the current ALL-2008 protocol and to implementation of a treatment stratification primarily based on cytogenetics and early MRD monitoring. We here present the results of the NOPHO ALL-92 and ALL-2000 studies and our current strategy for treatment of childhood ALL. In recent years, infants with ALL have been treated according to the Interfant protocols,[20] and patients with translocation t(9;22)(q34;q11) have been treated according to the international EsPhALL protocol for Philadelphia-positive childhood ALL.

Materials and methods

From January 1992 to December 2007, 2668 children 1.0–14.9 years of age were diagnosed with B-cell precursor or T-cell ALL in the Nordic countries and enrolled in the ALL-92 protocol (1992 to 2001, $N=1645$) or the ALL-2000 protocol (2002 to 2007, $N=1023$). The diagnosis of ALL was based on morphologic evaluation of bone marrow smears in combination with immunophenotyping with panels of monoclonal antibodies directed toward lineage-associated antigens. Patients with mature B-ALL have been treated according to mature B-cell non-Hodgkin lymphoma protocols, and they are excluded from this report. Only G-band karyotyping was mandatory in the ALL-

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Table 1 Treatment outcome according to NOPHO ALL study periods

NOPHO protocol periods *Year*	*Non-uniform* *1981–1985*	*Non-uniform* *1986–1991*	*ALL-92* *1992–2001*	*ALL-2000* *2002–2007*
No. of patients	719	937	1645	1023
Induction death[a]	36	16	21	16
Resistant disease[a]			9	14
Complete remission	375	627	1223	848
Relapses				
Hematologic only	145	164	214	80
CNS only	62	35	44	24
Hematologic+CNS	42	23	33	14
Testicular only	23	20	14	2
Hematologic+testis	8	10	15	0
Hematologic+CNS+testis	2	1	1	0
Other relapses sites	5	11	14	6
Second cancer	6	15	21	4
Death in first remission	13	14	34	15
10-year cumulative remission death	0.02 ± 0.01	0.01 ± 0.0	0.02 ± 0.0	—
5-year EFS ± s.e.	0.56 ± 0.02	0.70 ± 0.02	0.77 ± 0.01	0.79 ± 0.02
10-year EFS ± s.e.	0.53 ± 0.02	0.68 ± 0.02	0.75 ± 0.01	—
15-year EFS ± s.e.	—	—	0.74 ± 0.01	—
5-year overall survival ± s.e.	0.73 ± 0.02	0.82 ± 0.01	0.88 ± 0.01	0.89 ± 0.01
10-year overall survival ± s.e.	0.68 ± 0.02	0.78 ± 0.01	0.85 ± 0.01	—
15-year overall survival ± s.e.	—	—	0.84 ± 0.01	—

Abbreviations: ALL, acute lymphoblastic leukemia; CNS, central nervous system; EFS, event-free survival; NOPHO, Nordic Society of Paediatric Haematology and Oncology; s.e., standard error.
[a]No uniform definition of resistant disease before 1992 because of lack of uniform treatment strategies. Before 1992, the figures refer to patients who died before remission. After 1992, resistant disease encompasses those that obtained remission only after shifting to alternative treatment protocols.

92 protocol, but the ALL-2000 protocol also required directed analysis by fluorescent *in situ* hybridization and/or reverse transcriptase PCR for translocations t(9;22)(q34;q11)(*BCR–ABL*) or t(1;19)(q23;p13)(*E2A–PBX1*), and for 11q23/*MLL* aberrations. Furthermore, many leukemic samples have been examined by comparative genomic hybridization, spectral karyotyping and DNA-index by flow cytometry, and nearly all patients have in recent years been examined for the t(12;21)(*ETV6–RUNX1*) translocation, although the presence of this translocation does not influence the treatment stratification.[9] All cytogenetic results are scrutinized annually by the NOPHO cytogenetic working group and described according to ISCN 1995.[21] The protocols were approved by the regional or national ethics committees, and informed consent was obtained according to the Declaration of Helsinki.

Risk grouping and treatment

NOPHO ALL-92 risk grouping

Details of the NOPHO ALL-92 protocol have been described in previous publications.[4,6,22] The risk group assignment was based on age and white blood cell count (WBC) at diagnosis (standard risk (SR): age 2.0–9.9 years and WBC $<10.0\times10^9$/l; intermediate risk (IR): age 1.0–1.9 or $\geqslant$10.0 years and/or WBC 10–49.9×10^9/l; higher risk (that is, HR or very high risk (VHR)): WBC$\geqslant50.0\times10^9$/l) and the presence of higher risk features: T-lineage ALL, the presence of CNS or testicular involvement, translocations t(9;22)(q34;q11) or t(4;11)(q21;q23), lymphomatous leukemia or mediastinal lymphoma, and/or a poor treatment response (M3 BM at day 15 or M2/M3 at day 29).[4] Patients who had higher risk features were assigned to the VHR treatment arm, if they were at least 5 years of age at diagnosis (because of the use of cranial irradiation in that protocol arm) and in addition had (i) T-cell disease with one or more additional HR-features, (ii) CNS leukemia, (iii) lymphomatous leukemia and/or (iv) higher risk ALL at diagnosis and a day 15 M3 or a day 29 M2/M3 bone marrow.

NOPHO ALL-92 therapy

Induction therapy. All patients received Prednisolone (60 mg/m^2/day on days 1–36, then tapered), weekly VCR (2.0 mg/m^2 six times, maximum 2.0 mg), Doxorubicin (40 mg/m^2 three times (SR and IR) or four times (HR)), Erwinia asparaginase (30.000 IU/m^2 daily on days 37–46) and intrathecal (i.t.) MTX on four occasions.

Early intensification. Immediately after induction therapy, IR- and HR-patients received two doses of Cyclophosphamide (1000 mg/m^2 two times, 4 weeks apart) with low-dose Cytarabine (75 mg/m^2 daily for two 4-day periods after each Cyclophosphamide dose) and oral 6MP.

Consolidation. For SR–ALL, consolidation therapy included three courses of high-dose MTX (HD–MTX) 5 g/m^2/24 h with i.t. MTX and Leucovorin rescue. Patients with IR–ALL received oral 6MP (25 mg/m^2/day) with four courses of HD–MTX 5 g/m^2/24 h with i.t. MTX and Leucovorin rescue at 2 weeks intervals, whereas patients with HR– or VHR–ALL received HD–MTX 8 g/m^2/24 h with i.t. MTX and Leucovorin rescue, alternating with high-dose Cytarabine (12 g/m^2) two times (VHR) or four times (HR) with two 2-month intervening periods of oral weekly MTX and daily 6MP with two VCR/Prednisolone reinductions per period.

Delayed intensification. Patients with IR-, HR- or VHR-ALL received delayed intensification with dexamethasone (10 mg/m^2/day for 3 weeks, then tapered), weekly VCR (2.0 mg/m^2 four times), weekly anthracycline (30 mg/m^2/day Doxorubicin three times (HR) or daunorubicin four times (IR)) and Erwinia asparaginase (30.000 IU/m^2 four times)[4] followed by Cyclophosphamide 1000 mg/m^2, low-dose Cytarabine and 6-Thioguanine.

6-Mercaptopurine/methotrexate maintenance therapy. This therapy was initiated at treatment weeks 13 (SR), 32 (IR) or 63 (HR) and continued until 2 (IR and HR) or $2\frac{1}{2}$ years (SR) after diagnosis. During the first year of maintenance therapy, patients with SR– or IR–ALL received alternate pulses at 4-week intervals of (i) VCR (2.0 mg/m^2 once) and Prednisolone (60 mg/m^2/day for 1 week) and (ii) HD–MTX 5 g/m^2/24 h with i.t. MTX and Leucovorin rescue until five courses of HD–MTX had been given. Every 8 weeks throughout maintenance therapy, HR patients received reinductions of VCR (1.5 mg/m^2 once) and Prednisolone (40 mg/m^2/day for 5 days) with i.t. MTX.

Hematopoietic stem cell transplantation (SCT). There were no uniform Nordic criteria for SCT in the NOPHO ALL-92 protocol. In total, 57 patients (3.5%) underwent SCT in first remission.

In addition to the inclusion of cranial irradiation for patients with VHR–ALL, the primary therapeutic difference between the HR and VHR protocol was the substitution of oral MTX/6MP maintenance therapy (HR) with cyclic LSA_2L_2 maintenance therapy (VHR).[4,7] Furthermore, to examine whether a more intensive cyclic multi-drug maintenance therapy regimen could reduce the relapse rate for patients with higher risk features, Finnish patients with higher risk features received LSA_2L_2 maintenance therapy irrespective of whether their induction/consolidation/delayed intensification/CNS-directed therapy had been according to the HR– or VHR–ALL regimens.[7,23]

Between 1992 and 1996, 538 patients with SR–, IR– or HR–ALL entered into the randomized ALL-92 oral MTX/6MP maintenance therapy trial. It examined the prognostic effect of pharmacologically guided dose adjustments of oral 6MP/MTX maintenance therapy by a combination of a target degree of leukopenia and the erythrocyte levels of 6-Thioguanine nucleotides (cytotoxic metabolites of 6MP) and MTX-polyglutamates (the cytotoxic metabolites of MTX).[6]

NOPHO ALL-2000 risk grouping

The risk assignment features were very similar to that of the ALL-92 protocol, except that (i) all children aged 1.0–9.9 years were eligible to the SR-arm, if their WBC was $<10\times10^9$/l and they had no HR group features, (ii) t(1;19)(q23;p13), hypodiploidy (<45 chromosomes) and all *MLL*-rearrangements were included in higher risk features, and (iii) the higher risk group patients were stratified into three treatment arms, that is, HR, VHR and extra HR. Thus, patients who had higher risk features were assigned to the VHR treatment arm, if they were at least 5 years of age at diagnosis (because of the use of cranial irradiation in that protocol arm) and in addition had WBC of $100–199\times10^9$/l, and/or T-cell disease with mediastinal mass and/or CNS leukemia. Patients with WBC $\geqslant 200\times10^9$/l, *MLL*-rearrangement and age >10 years, hypodiploidy <34 chromosomes, translocation t(9;22)(q34;q11), and/or a M3 bone marrow day 29, were stratified to the extra HR group and offered allogeneic SCT in first complete remission. Furthermore, it was optional to offer SCT in first remission to patients with MRD levels $\geqslant 10^{-3}$ after 3 months of antileukemic therapy. All other patients with higher risk features were stratified to the HR–ALL group. In total, 62 patients (6.1%) were treated with SCT in first remission.

NOPHO ALL-2000 therapy

The NOPHO ALL-2000 therapy was a modification of the ALL-92 treatment strategy.

Induction therapy was identical to that of the ALL-92 protocol except that (i) one dose less of Doxorubicin was given, (ii) the ceiling dose of VCR was set to 2.5 mg and (iii) Erwinase was substituted with *Escherichia coli* asparaginase (6.500 IU/ at 3–4 days intervals, four times).

Consolidation therapy. The SR– and IR–ALL patients received identical consolidation therapy starting treatment day 50 with 6MP (25 mg/m^2/day) and alternating blocks with either HD–MTX 5 g/m^2/24 h with i.t. MTX (age adjusted) and Leukovorin rescue 15 mg/m^2 (three times) or low-dose cytarabine (75 mg/m^2/day for 4 days, two times), whereas the consolidation therapy for the higher risk patients was identical to that of the ALL-92 protocol.

Delayed intensification. Patients with IR–, HR– or VHR–ALL received delayed intensification similar to that of the ALL-92 protocol except that (i) dexamethasone was given for 2 weeks only (IR: 6 mg, higher risk 10 mg/m^2/day), (ii) the maximum dose of VCR was set to 2.5 mg and (iii) Erwinase was substituted with *E. coli* asparaginase (6.500 IU/ at 3–4 days intervals, four times).

Irradiation. Before maintenance therapy, children above 5 years of age, who were allocated to the VHR group, received 18-Gy cranial irradiation (or 24 Gy in case of CNS leukemia at diagnosis) with i.t. MTX weekly (three times) and oral 6MP 75 mg/m^2. The dose of 6MP was reduced to 25 mg/m^2 or to 5–10 mg/m^2 in case of one or two thioupurine methyltransferase (TPMT) low activity alleles, respectively.[24]

LSA_2L_2 therapy. Courses of LSA_2L_2 therapy, identical to those used in the NOPHO ALL-92 protocol were given two times (HR) or three times (VHR) before the start of MTX/6MP maintenance therapy, or until SCT could be performed (VHR–ALL).

Classical oral 6MP/MTX maintenance therapy. This therapy was initiated at treatment weeks 17 (SR), 30 (IR), 70 (HR) or 61 (VHR) and continued until 2 (HR and VHR) or $2\frac{1}{2}$ years (SR and IR) from diagnosis. The starting dose of oral MTX was 20 mg/m^2, and the dose of oral 6MP was adjusted according to the TPMT activity being 75 mg/m^2/day for wild-type patients, 50 mg/m^2/day for heterozygous patients and 5–10 mg/m^2/day for TPMT deficient patients. The doses of MTX and 6MP was adjusted to a target WBC of $1.5–3.5\times10^9$/l. During the first year of MTX/6MP maintenance therapy, patients with SR– or IR–ALL received alternate pulses at 4-week intervals of (i) VCR (2.0 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 h with i.t. MTX and Leucovorin rescue until five courses of HD–MTX had been given. Every 4 weeks throughout 6MP/MTX maintenance therapy, HR and VHR patients received reinductions of VCR (2.0 mg/m^2 once, maximum 2.5 mg) and dexamethasone (6 mg/m^2/day for 5 days).

After the first year of maintenance therapy patients with SR– or IR–ALL were randomized to either none or eight further

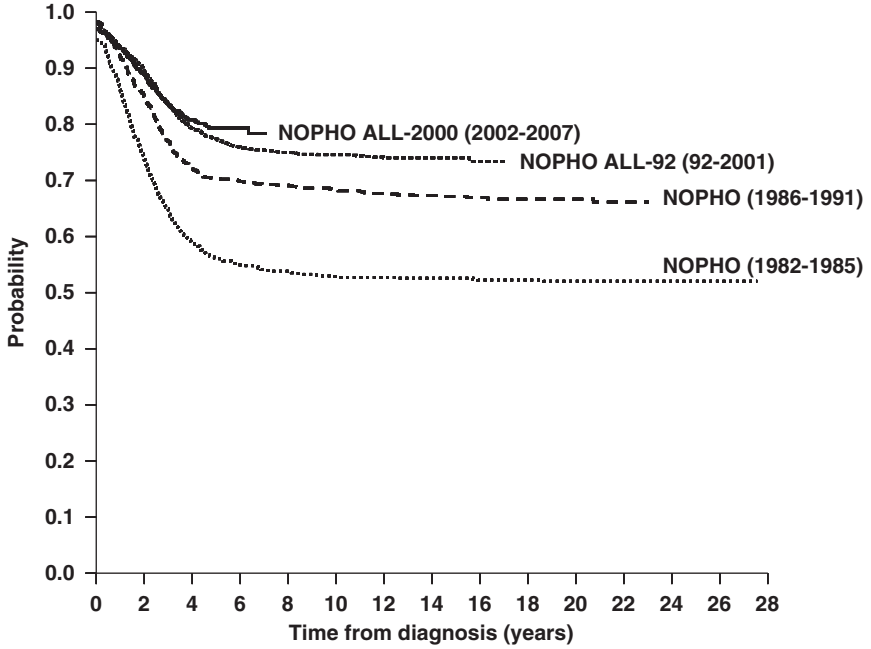

Figure 1 Event-free survival (EFS) in four consecutive Nordic Society of Paediatric Haematology and Oncology (NOPHO) cohort periods.

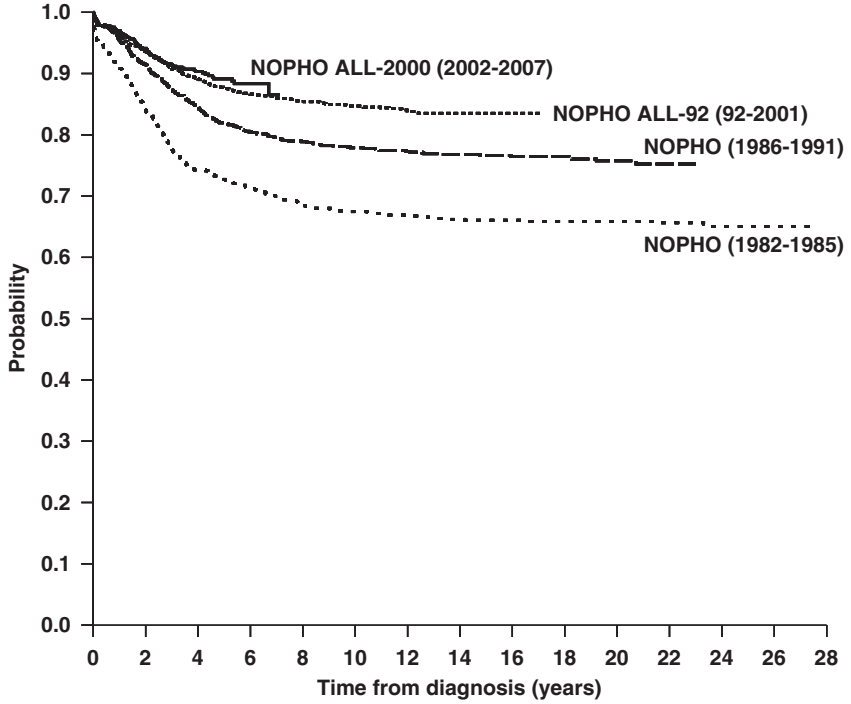

Figure 2 Overall survival in four consecutive Nordic Society of Paediatric Haematology and Oncology (NOPHO) cohort periods.

reinductions with VCR (2.0 mg/m^2 once, maximum 2.5 mg) and dexamethasone (6 mg/m^2/day for 5 days) at 6 weeks interval. This study is still open for patient accrual and study results are not reported here.

Statistical analysis

Survival analyses were performed with a basic time scale defined by the date of diagnosis. The duration of event-free survival (EFS) was defined as the time from diagnosis until the date of induction failure, relapse, death or the development of a second malignancy (whichever first) or the last known follow-up for event-free survivors. Cases that did not attain a complete remission were considered failures at time zero. For patients who achieved complete remission, the cumulative risk of any relapse, of isolated CNS or any (isolated plus combined) CNS relapse, testicular relapse, therapy-related second malignancy or of toxic death were estimated by the method of Kalbfleisch and

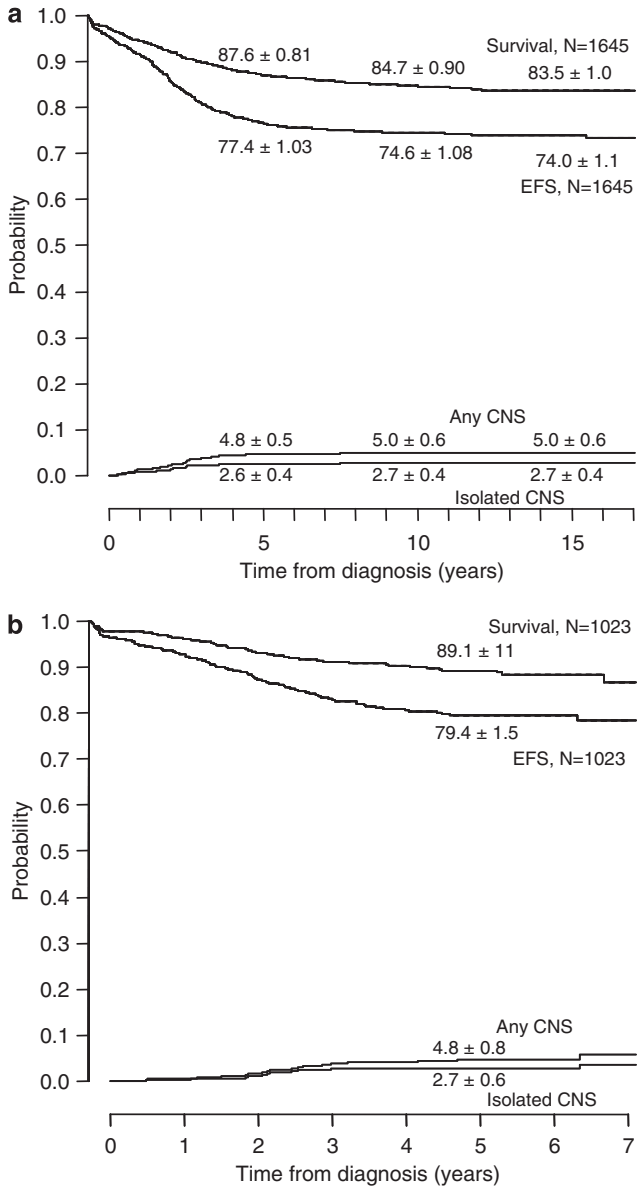

Figure 3 (**a**) Event-free survival (EFS), survival and cumulative incidence of isolated or any central nervous system (CNS) relapse in Nordic Society of Paediatric Haematology and Oncology (NOPHO) acute lymphoblastic leukemia (ALL)-92 study. (**b**) EFS, survival and cumulative incidence of isolated or any CNS relapse in NOPHO ALL-2000 study.

Prentice, and outcomes were compared with Gray's test, adjusting for competing events. An isolated CNS relapse was defined as a CNS relapse without relapse at other sites. A CNS relapse included any relapse with CNS involvement. Cox proportional hazards regression analyses were performed to identify independent prognostic factors for differences in outcome.[25,26] Non-parametric methods were applied to compare the distribution of parameters between subgroups.[27] The Kaplan–Meier method was applied for estimation of remission duration and for the generation of survival curves.[28] Two-sided *P*-values <0.05 were regarded as significant. The status of each patient in the NOPHO leukemia registry is updated at least annually. Within 10 years from the diagnosis of ALL (median: 6.7 years) 18 patients from the ALL-92 cohort were lost to follow-up in the NOPHO ALL registry because of emigration outside the Nordic countries ($N=3$, median: 2.8 years), change of Nordic address ($N=4$, median: 5.2 years), transfer to an adult department ($N=5$, median: 6.8 years) or cessation of clinical control or not otherwise specified ($N=6$, median: 7.9 years). Similarly, a total of eight patients from the ALL-2000 cohort were lost to follow-up in the NOPHO ALL registry protocol at a median of 2.5 years from diagnosis.

Results

Compared with the Nordic 10-years EFS results for the 1981–1985 and 1986–1991 cohorts (52.9 and 68.3%, respectively), the EFS improved significantly in the ALL-92 protocol (74.6 ± 1.1%) with indications of a further non-significant improvement in the ALL-2000 protocol (Table 1). Today failures very rarely occur beyond 10 years from diagnosis (<1%) (Figure 1).

Protocol-specific treatment outcome

The NOPHO ALL-92: at 10 years, the EFS was 74.6 ± 1.1% and the overall survival was 84.7 ± 0.9% for the 1645 evaluable patients enrolled (Figures 1–3). The cumulative risk estimates for isolated CNS and any CNS relapses were 2.6 ± 0.4% and 4.8 ± 0.5% at 5 years and 2.7 ± 0.4% and 5.0 ± 0.6% at 10 years, respectively (Figure 3). In spite of administration of CNS irradiation, the cumulated risk of CNS relapse (combined or isolated) among VHR–ALL patients was 9.3 ± 2.3% with seven of eight such cases having T-cell ALL and all being males.

Of the 889 male patients, 32 developed a testicular relapse (isolated or combined with other relapse sites in 14 and 18 patients, respectively). The 5-year and 10-year risks for isolated testicular relapse were both 0.7 ± 0.2%.

Patients who relapsed had higher average neutrophil counts (median: 2.2 vs 1.9×10^9/l, $P=0.0008$) and WBC during maintenance therapy (3.5 vs 3.3×10^9/l, $P=0.06$) than those who stayed in remission, but did not differ in their average lymphocyte counts ($P=0.60$).[6] For both sexes, patients with an average neutrophil count during maintenance therapy of $<2.0\times10^9$/l (median of all patients) had an EFS superior to that of patients with higher average neutrophil counts (boys: 0.87 vs 0.75, $P=0.02$, girls: 0.94 vs 0.83, $P=0.01$).[6] Furthermore, the risk of relapse was higher for the patients who received MTX/6MP maintenance therapy and had normal TPMT activity compared with those with reduced TPMT activity (18 vs 7%, respectively $P=0.03$).[5] Patients stratified to pharmacologically adjusted 6MP/MTX maintenance therapy had an increased risk of relapse compared with those in the conventionally treated control arm, however, this was only statistically significant for girls (0.18 ± 0.03 vs 0.05 ± 0.02, $P=0.002$).[6]

For patients with HR– or VHR–ALL, administration of LSA_2L_2 maintenance therapy was related to a significantly increased risk of relapse compared with that obtained with oral 6MP/MTX maintenance therapy.[7]

In Cox multivariate regression analysis that also included gender, age, WBC at diagnosis, immunophenotype and the randomization groups, the average degree of myelosuppression during maintenance therapy was significantly related to the risk of relapse.[6] Thus, patients who's average leukocyte level during maintenance therapy was below 3.5×10^9/l (protocol target) had a significantly lower risk of relapse than the patients with higher average leukocyte counts (0.14 ± 0.02 vs 0.21 ± 0.03, $P=0.03$).

Table 2 Treatment results according to presenting features of patients treated in NOPHO ALL-92 study

Factors	*No. of patients*	*Event-free survival ± s.e. (%)*				*Overall survival ± s.e. (%)*			
		Year 5	*Year 10*	*Year 15*	P-*value*	*Year 5*	*Year 10*	*Year 15*	P-*value*
B-lineage									
NCI standard	1093	0.84 ± 0.01	0.81 ± 0.01	0.80 ± 0.01	<0.001	0.93 ± 0.01	0.91 ± 0.01	0.89 ± 0.01	<0.001
NCI high	376	0.65 ± 0.03	0.63 ± 0.03	0.63 ± 0.03		0.81 ± 0.02	0.76 ± 0.02	0.75 ± 0.02	
T-lineage									
NCI standard	35	0.69 ± 0.08	0.69 ± 0.08	0.69 ± 0.08	0.44	0.74 ± 0.07	0.74 ± 0.07	0.74 ± 0.07	0.362
NCI high	117	0.59 ± 0.05	0.59 ± 0.05	0.59 ± 0.05		0.64 ± 0.04	0.64 ± 0.04	0.64 ± 0.04	
Sex									
Male	889	0.75 ± 0.01	0.72 ± 0.02	0.71 ± 0.02	0.009	0.87 ± 0.01	0.84 ± 0.01	0.82 ± 0.01	0.168
Female	756	0.80 ± 0.02	0.78 ± 0.02	0.77 ± 0.02		0.88 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	
Age at diagnosis (years)									
1–9	1378	0.79 ± 0.01	0.76 ± 0.01	0.75 ± 0.01	0.004	0.89 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	<0.001
>10	267	0.70 ± 0.03	0.68 ± 0.03	0.68 ± 0.03		0.82 ± 0.02	0.77 ± 0.03	0.76 ± 0.03	
WBC × 10^9/l									
<10	838	0.82 ± 0.01	0.78 ± 0.01	0.78 ± 0.01	<0.001	0.91 ± 0.01	0.88 ± 0.01	0.87 ± 0.01	<0.001
10–49	516	0.81 ± 0.02	0.79 ± 0.02	0.77 ± 0.02		0.91 ± 0.01	0.88 ± 0.01	0.86 ± 0.02	
50–99	139	0.66 ± 0.04	0.65 ± 0.04	0.64 ± 0.04		0.79 ± 0.03	0.75 ± 0.04	0.75 ± 0.04	
>100	152	0.49 ± 0.04	0.49 ± 0.04	0.49 ± 0.04		0.65 ± 0.04	0.62 ± 0.04	0.60 ± 0.04	
Cell lineage									
BCP	1469	0.79 ± 0.01	0.76 ± 0.01	0.76 ± 0.01	<0.001	0.90 ± 0.01	0.87 ± 0.01	0.85 ± 0.01	<.0001
T	152	0.61 ± 0.04	0.61 ± 0.04	0.61 ± 0.04		0.66 ± 0.04	0.66 ± 0.04	0.66 ± 0.04	
CNS status									
CNS 1+2	1607	0.77 ± 0.01	0.75 ± 0.01	0.74 ± 0.01	0.005	0.88 ± 0.01	0.85 ± 0.01	0.83 ± 0.01	0.001
CNS 3	37	0.60 ± 0.08	0.60 ± 0.08	0.60 ± 0.08		0.70 ± 0.08	0.68 ± 0.08	0.67 ± 0.08	
HeH; >50 chr									
Yes	410	0.81 ± 0.02	0.78 ± 0.02	0.78 ± 0.02	0.001	0.89 ± 0.02	0.87 ± 0.02	0.87 ± 0.02	0.03
No	540	0.71 ± 0.02	0.69 ± 0.02	0.69 ± 0.02		0.85 ± 0.02	0.81 ± 0.02	0.79 ± 0.02	
Unknown	695	0.80 ± 0.02	0.77 ± 0.02	0.76 ± 0.02		0.89 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	
DNA index									
>1.10	28	0.75 ± 0.08	0.71 ± 0.09	0.71 ± 0.09	ns	0.93 ± 0.05	0.85 ± 0.07	0.85 ± 0.07	ns
⩽1.10	33	0.70 ± 0.08	0.66 ± 0.09	0.66 ± 0.09		0.82 ± 0.07	0.78 ± 0.08	0.78 ± 0.08	
Unknown	1584	0.77 ± 0.01	0.75 ± 0.01	0.74 ± 0.01		0.88 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	
t(9;22)									
Present	32	0.44 ± 0.09	0.41 ± 0.09	0.41 ± 0.09	<0.001	0.59 ± 0.09	0.56 ± 0.09	0.56 ± 0.09	<0.001
Absent	919	0.76 ± 0.01	0.74 ± 0.01	0.74 ± 0.01		0.88 ± 0.01	0.85 ± 0.01	0.83 ± 0.01	
Unknown	694	0.80 ± 0.02	0.77 ± 0.02	0.76 ± 0.02		0.89 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	
t(1;19)									
Present	21	0.81 ± 0.09	0.81 ± 0.09	0.81 ± 0.09	ns	0.95 ± 0.05	0.90 ± 0.07	0.90 ± 0.07	ns
Absent	930	0.75 ± 0.01	0.73 ± 0.01	0.72 ± 0.01		0.87 ± 0.01	0.84 ± 0.01	0.82 ± 0.01	
Unknown	694	0.80 ± 0.02	0.77 ± 0.02	0.76 ± 0.02		0.89 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	
t(12;21)									
Present	174	0.80 ± 0.03	0.76 ± 0.03	0.75 ± 0.04	ns (0.06)	0.94 ± 0.02	0.90 ± 0.02	0.83 ± 0.05	0.01
Absent	589	0.73 ± 0.02	0.72 ± 0.02	0.72 ± 0.02		0.85 ± 0.01	0.82 ± 0.02	0.81 ± 0.02	
Unknown	882	0.79 ± 0.01	0.76 ± 0.01	0.75 ± 0.01		0.88 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	
t(4;11)									
Present	4	0.25 ± 0.22	0.25 ± 0.22	NA	<0.01	0.25 ± 0.22	0.35 ± 0.22	NA	0.001
Absent	947	0.75 ± 0.01	0.73 ± 0.01	0.73 ± 0.01		0.87 ± 0.01	0.84 ± 0.01	0.83 ± 0.01	
Unknown	694	0.80 ± 0.02	0.77 ± 0.02	0.76 ± 0.02		0.89 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	

Abbreviations: ALL, acute lymphoblastic leukemia; BCP, B-cell precursor; CNS, central nervous system; HeH, high-hyperdiploid leukemia; MRD, minimal residual disease; NCI, National Cancer Institute risk assignment criteria; NOPHO, Nordic Society of Paediatric Haematology and Oncology; s.e., standard error; WBC, white blood cell count.
Outcome in ALL subsets defined by clinical or laboratory parameters.

So far 21 second malignant neoplasms have been encountered on the ALL-92 protocol of which 16 were myelodysplasias or acute myeloid leukemias.[29] The cumulative risk of any second malignant neoplasm was 1.1 ± 0.3% at 10 years and 1.2 ± 0.4% at 15 years, and that of second myeloid malignancies plateaued at 1.1 ± 0.3% at 7.5 years. The risk of developing a

Table 3 Treatment results according to presenting features of patients treated in NOPHO ALL-2000 study

Factors	*No. of patients*	*Event-free survival ± s.e. (%)*				*Overall survival ± s.e. (%)*			
		Year 5	*Year 10*	*Year 15*	P-*value*	*Year 5*	*Year 10*	*Year 15*	P-*value*
B-lineage									
NCI standard	645	0.85 ± 0.02	—	—	<0.001	0.95 ± 0.01	—	—	<0.001
NCI high	261	0.72 ± 0.03	—	—		0.82 ± 0.03	—	—	
T-lineage									
NCI standard	31	0.73 ± 0.08	—	—	0.24	0.79 ± 0.08	—	—	0.378
NCI high	84	0.61 ± 0.05	—	—		0.69 ± 0.05	—	—	
Sex									
Male	569	0.78 ± 0.02	—	—	0.36	0.89 ± 0.02	—	—	0.915
Female	454	0.81 ± 0.02	—	—		0.90 ± 0.02	—	—	
Age at diagnosis (years)									
1–9	840	0.81 ± 0.02	—	—	0.001	0.91 ± 0.01	—	—	<0.001
>10	183	0.71 ± 0.04	—	—		0.80 ± 0.04	—	—	
WBC $\times 10^9$*/l*									
<10	154	0.84 ± 0.02	—	—	<0.001	0.94 ± 0.01	—	—	0.0001
10–49	109	0.80 ± 0.03	—	—		0.90 ± 0.02	—	—	
50–99	30	0.74 ± 0.05	—	—		0.84 ± 0.04	—	—	
>100	65	0.63 ± 0.05	—	—		0.70 ± 0.04	—	—	
Cell lineage									
BCP	906	0.81 ± 0.02	—	—	<0.001	0.91 ± 0.01	—	—	<0.001
T	115	0.64 ± 0.05	—	—		0.72 ± 0.0	—	—	
CNS status									
CNS 1+2	990	0.80 ± 0.02	—	—	0.004	0.90 ± 0.01	—	—	0.018
CNS 3	30	0.63 ± 0.10	—	—		0.77 ± 0.09	—	—	
HeH (>50 chr)									
Yes	321	0.84 ± 0.02			0.08	0.93 ± 0.02			ns
No	473	0.76 ± 0.02				0.87 ± 0.02			
Unknown	229	0.80 ± 0.03				0.89 ± 0.02			
DNA index									
>1.10	43	0.76 ± 0.08			ns	0.90 ± 0.05			ns
⩽1.10	93	0.76 ± 0.05				0.88 ± 0.04			
Unknown	887	0.80 ± 0.02				0.89 ± 0.01			
t(9;22)									
Present	9	0.44 ± 0.17			0.01	0.78 ± 0.14			ns
Absent	786	0.79 ± 0.02				0.89 ± 0.01			
Unknown	228	0.82 ± 0.03				0.89 ± 0.02			
t(1;19)									
Present	26	0.82 ± 0.08			ns	0.87 ± 0.07			ns
Absent	769	0.79 ± 0.02				0.89 ± 0.01			
Unknown	228	0.81 ± 0.03				0.89 ± 0.02			
t(12;21)									
Present	201	0.86 ± 0.03			<0.001	0.96 ± 0.02			<0.001
Absent	666	0.77 ± 0.02				0.88 ± 0.01			
Unknown	256	0.81 ± 0.04				0.86 ± 0.03			
t(4;11)									
Present	9	0.20 ± 0.17			<0.001	0.36 ± 0.19			<0.001
Absent	785	0.80 ± 0.02				0.90 ± 0.01			
Unknown	229	0.80 ± 0.03				0.89 ± 0.02			
MRD day 29									
⩾5%	55	0.45 ± 0.07			<0.001	0.60 ± 0.08			<0.001
⩾0.1–<5%	195	0.74 ± 0.04				0.90 ± 0.03			
<0.1%	497	0.86 ± 0.02				0.93 ± 0.01			
No MRD analysis	276	0.77 ± 0.01				0.87 ± 0.01			

Abbreviations: ALL, acute lymphoblastic leukemia; BCP, B-cell precursor; CNS, central nervous system; HeH, high-hyperdiploid leukemia; MRD, minimal residual disease; NCI, National Cancer Institute risk assignment criteria; NOPHO, Nordic Society of Paediatric Haematology and Oncology; s.e., standard error; WBC, white blood cell count.
Outcome in ALL subsets defined by clinical or laboratory parameters.

Table 4 Risk grouping in NOPHO ALL-2008 study

	Day 29 MRD			*Day 79 MRD*	
	<0.1%	*0.1–4.9%*	*⩾5%*	*<0.1%*	*⩾0.1%*
BCP and WBC <100	SR	IR	HR+SCT	As day 29 risk group	HR+SCT
T or WBC⩾100	IR	HR	HR+SCT	As day 29 risk group	HR+SCT
t(1;19)(q23;p13)	IR	IR	HR+SCT	As day 29 risk group	HR+SCT
dic(9;20)(p13;q11)	IR	IR	HR+SCT	As day 29 risk group	HR+SCT
ic21amp	IR	IR	HR+SCT	As day 29 risk group	HR+SCT
CNS3	IR	IR	HR+SCT	As day 29 risk group	HR+SCT
Biphenotypic	IR	IR	HR+SCT	As day 29 risk group	HR+SCT
Hypodiploidy<45	HR	HR	HR+SCT	As day 29 risk group	HR+SCT
MLL rearranged	HR	HR	HR+SCT	As day 29 risk group	HR+SCT

Abbreviations: ALL, acute lymphoblastic leukemia; BCP, B-cell precursor; CNS, central nervous system; HR, high risk; IR, intermediate risk; MRD, minimal residual disease; NOPHO, Nordic Society of Paediatric Haematology and Oncology; SCT, hematopoietic stem cell transplantation in first remission; SR, standard risk; SR/IR/HR, standard, intermediate, high risk ALL; T-ALL, T-cell ALL; T/BCP, T-cell/B-cell precursor ALL; WBC, white blood cell count.
MRD will be monitored by flow cytometry for BCP–ALL and by PCR (clonal antigen receptor rearrangements) for T-ALL; T/BCP; WBC $\times 10^9$/l; SR/IR/HR; and SCT. The 4-week induction therapy with Vincristine, Doxorubicin, i.t. Methotrexate and a glucocorticosteroid is identical for all patients except that patients with T-lineage and/or leukocyte count $\geqslant 100 \times 10^9$/l receive Dexamethasone 10 mg/m^2/day for 3 weeks and all other patients receive Prednisolone 60 mg/m^2/day for 4 weeks. Translocation t(1;19)(q23;p13), dic(9;20)(p13;q11), intrachromosomal amplification of chromosome 21,[44] CNS leukemia (=CNS3) at diagnosis, and bi-lineage or bi-phenotypic ALL always stratify patients to the IR group, unless HR features are present. Hypodiploid ALL (<45 chromosomes) and MLL rearrangements always stratify patients to the HR group. 55% of all patients are projected to be allocated to the SR group, 35% to the IR group, and 10% to the HR group of whom half are projected to receive a SCT in first remission. SR and IR patients will receive conventional ALL therapy, whereas HR–ALL patients as part of their treatment will receive nine very intensive treatment blocks. The total duration of therapy will be 2.5 years for all non-SCT patients.

second malignant neoplasm was significantly related to oral MTX/6MP maintenance therapy characteristics such as longer duration of maintenance therapy, higher oral 6MP doses and lower activity of TPMT, which leads to higher intracellular levels of the cytotoxic 6-thioguanine nucleotides.[29] The risk of second malignant neoplasm was inversely related to the risk group, being 2.4 ± 0.7% for SR–ALL, 1.2 ± 0.7% for IR–ALL and 0.3 ± 0.3% for HR–ALL patients ($P=0.007$).[29]

NOPHO ALL-2000. At 5 years, the EFS was 79.4 ± 1.5% and the overall survival was 89.1 ± 1.1% for the 1023 evaluable patients enrolled between 2002 and 2007 (Figures 1–3). The cumulative risk estimates for isolated CNS and any CNS relapses were 2.7 ± 0.6 and 4.8 ± 0.8% at 5 years (Figure 3). Of the 569 male patients only two have developed a testicular relapse, both in combination with hematological relapse, with an overall 5-year risk for testicular relapse of 0.2 ± 0.2%. The randomized study on the efficacy of VCR/dexamethasone will accrue patients until mid 2009 and the results are still blinded.

Treatment results according to presenting features

Prognostic factors present at diagnosis were examined in combined analyses of the NOPHO ALL-92 and ALL-2000 studies. High-risk B-lineage according to the NCI/Rome criteria (age <1 or >10 years with leukocyte count $>50 \times 10^9$/l) and T-cell phenotype were adverse prognostic factors during both study periods (Tables 2 and 3).[30]

Male gender was associated with a reduced EFS in the ALL-92 protocol (75.4 vs 79.9 &, $P=0.009$), whereas in the ALL-2000 protocol the outcome did not differ significantly for boys and girls.

In univariate analysis, CNS disease at diagnosis (defined as $\geqslant 5 \times 10^6$/l leukemic cells in the diagnostic spinal tap) was related to a significantly increased risk of treatment failure ($P<0.01$ in both study cohorts), but that was not the case if the Cox regression analysis included T-cell immunophenotype and WBC at diagnosis ($P>0.05$ in both the ALL-92 and ALL-2000 cohorts).

The *ETV6–RUNX1* translocation was linked to a favorable prognosis in both the ALL-92 ($P<0.001$) and the ALL-2000 protocol ($P=0.06$), primarily because of its strong association with lower age and low leukocyte count,[9,11] and it has not been included in the risk grouping in the previous NOPHO studies or the current NOPHO ALL-2008 protocol.

Except for MRD levels $\geqslant 10^{-3}$ after 3 months of antileukemic therapy being an optional indication for SCT in first remission in the ALL-2000 study, MRD has not been used for treatment stratification in the ALL-92 or the ALL-2000 studies. The ALL-2000 study confirmed that the post-induction (day 29) MRD levels is a strong independent prognostic factor for both EFS and overall survival [16] (Table 3).

Discussion

The collaboration on risk-adapted treatment of childhood ALL has within the last decades yielded almost identical survival rates for the five Nordic countries (data not shown), which overall now reach 88% at 5 years and 85% at 10 years. Later events have become very rare, and non-CNS extramedullary relapses now account for <5% of all events. However, the overall recurrence rate of leukemia in the CNS, which was 4–5% in both study ALL-92 and study ALL-2000, calls for further improvement. The importance of CNS irradiation in first remission has been questioned,[31] and in a combined analysis of the ALL-92 and ALL-2000 studies, the incidence of CNS relapse was not significantly reduced, when CNS irradiation was given to patients at the highest risk of this event (age⩾10 years, T-cell ALL, and/or WBC $\geqslant 50 \times 10^9$/l at diagnosis) ($P=0.63$). To reduce the risk of sequelae, no patients in the current ALL-2008 study will be exposed to CNS irradiation during first remission, but we have added i.t. MTX at 8-week intervals during maintenance therapy for patient with IR features, and we will examine the efficacy and toxicity of i.t. Depocyte (liposomal Cytarabine) in a randomized study for HR–ALL patients.[32]

Childhood leukemia are driven by mutations,[33] but the prognostic significance of the cytogenetic aberrations depends

on the treatment given and thus vary between study groups. Some subsets such as translocation t(1;19)(q23;p13) and dic(9;20)(p13;q11) have a high *in vitro* chemosensitivity,[10,13] but may require relatively intensive chemotherapy to reduce their risk of relapse, and in the NOPHO ALL-2008 protocol these cytogenetic subsets are excluded from SR therapy. Other subsets such as those with 11q23/*MLL* aberrations or a hypodiploid clone (<45 chromosomes) do poorly in all studies[34,35] and should be offered very intensive chemotherapy. Whether the latter two subsets will gain from hematopoietic SCT remain to be shown, and in the NOPHO ALL-2008 protocol these will be offered chemotherapy only (Table 4).[35,36]

Monitoring of MRD by PCR for clonal immune-gene rearrangements or by flowcytometric identification of leukemic cells with aberrant antigen expression has emerged as one of the most powerful predictors of relapse.[16,37,38] It allows early modification of chemotherapy based on the *in vivo* response, and it has emerged as a powerful tool to determine the efficacy of early treatment phases.[37,39] Stratification of patients by MRD will allow identification of low-risk groups that can be cured by less intensive therapy as well as resistant patients that can be allocated to very intensive therapy with or without hematopoietic SCT. Although such stratification may improve the balance between cure rates and toxicities within the risk groups, it remains to be proven that this approach will improve the overall cure rates of childhood ALL.

The treatment of ALL involves application of a *specific* treatment protocol to a leukemic clone with *specific* mutations that has emerged in a patient with a *unique* geno- and phenotype. Accordingly, a large number of studies have shown that inherited nucleotide sequence variations in genes involved in absorption, metabolism, excretion, cellular transport and targets or target pathways of antileukemic agents significantly influence both the cure rate and individual risk of side effects.[40] Thus, in addition to randomized trials and mapping of mutations in the leukemic clones[33] exploration of genome sequence variations is expected to improve the understanding of the mechanisms for both treatment failures and life-threatening toxicities such as has been the case for polymorphisms in TPMT.[5,29] Accordingly, some studies have shown that the cure rates or the degree of toxicity can be influenced by individualization of therapy based on the patients drug disposition or previous degree of toxicity.[41,42]

One of the important challenges for hematologists is to improve the cure rates for adolescents and young adults to the level of that obtained for children.[43] To examine the biology of their leukemias, their pharmacology, treatment efficacy and pattern of side effects, as well as their compliance to therapy, adult hematologists in Denmark, Norway and Sweden will treat young adults between 18 and 45 years of age according to the NOPHO ALL-2008 protocol.

Conflict of interest

The authors declare no conflict of interest.

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