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Treatment stratification based on initial *in vivo* response in acute myeloid leukaemia in children without Down's syndrome: results of NOPHO-AML trials

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Summary. Three consecutive protocols for childhood acute myeloid leukaemia (AML) have been used in the Nordic countries since 1984: the Nordic Society for Paediatric Haematology and Oncology (NOPHO)-AML84 was of moderate intensity, NOPHO-AML88 of high intensity with upfront loading and aggressive consolidation. NOPHO-AML93 utilized the same treatment blocks as NOPHO-AML88, but after the first block those children with a hypoplastic non-leukaemic bone marrow were allowed to recover from aplasia. Poor responders received intensified induction therapy. Between January 1993 and December 2000, 219 children without Down's syndrome were entered on NOPHO-AML93. Compared with NOPHO-AML88, the event-free survival (EFS) at 7 years increased from 41% to 49% ($P = 0{\cdot}06$) and 7-year overall survival increased from 47% to 64% ($P < 0{\cdot}01$). Toxic death during induction was reduced from 10% to 3%. Survival was similar in patients receiving stem cell transplantation or chemotherapy only in first remission. The major prognostic factors in NOPHO-AML93 were response to therapy and cytogenetics. A total of 67% of patients achieved remission after the first induction course and showed an EFS of 56% compared with 35% in those not in remission ($P < 0{\cdot}01$). Cytogenetic results were obtained in 95% of patients. Patients with t(9;11) (p22;q23) ($n = 16$) experienced a significantly better EFS (86%) than other cytogenetic groups. The overall outcome was improved by employing the previous toxic protocol with different timings, and through individualizing therapy according to the initial response of the patient.

Keywords: acute myeloid leukaemia, children, cytogenetics, dose intensity, stem cell transplantation.

Childhood acute myeloid leukaemia (AML) is less common, more heterogeneous and has a poorer prognosis than that of acute lymphoblastic leukaemia (ALL) (Hurwitz *et al*, 1995; Lie, 1996; Lie *et al*, 1996; Ravindranath *et al*, 1996; Ritter, 1998; Zwaan *et al,* 2000). In recent years, therapeutic trials have shown that intensified induction and consolidation chemotherapy improves outcome. According to the best results published, up to half of all children with this disease are cured (Woods *et al*, 1996, 2001; Stevens *et al*, 1998; Creutzig *et al,* 2001; Webb *et al,* 2001; Perel *et al,* 2002). However, the therapy needs to be intensive, and the trend over the last decade has been to increase the intensity.

The Nordic Society for Paediatric Haematology and Oncology (NOPHO) comprises the countries Denmark, Finland, Iceland, Norway and Sweden. Since 1984, NOPHO has conducted population-based studies on children with AML, in three consecutive protocols. The first two studies, NOPHO-AML84 and NOPHO-AML88, showed an event-free survival (EFS) of 32% and 42% respectively (Lie *et al*, 1996). The studies included patients with Down's syndrome. NOPHO-AML84 was of modest intensity whereas this was intensified in NOPHO-AML88 by adding etoposide and mitoxantrone and reducing the interval between treatment courses. The toxicity of NOPHO-AML88 was

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significant with 14/118 deaths in aplasia during induction and 11/100 deaths in remission during consolidation (Lie *et al*, 1996).

NOPHO-AML93 used the same treatment courses as NOPHO-AML88, but the dose intensity was modified. Following the first course of induction, children without evidence of residual disease were allowed to recover before a second identical induction course. Children with persistent disease received sequentially different courses until remission was obtained.

We report the results of NOPHO-AML93 and compare them with those of NOPHO-AML88. The interval between the induction courses increased, the toxicity decreased and the overall survival improved.

PATIENTS AND METHODS

Patients. Between January 1988 and December 2000, 323 children with AML who were below 18 years of age, from the five Nordic countries, were treated on the two consecutive protocols NOPHO-AML88 ($n = 104$) and NOPHO-AML93 ($n = 219$). Patients with Down's syndrome, Fanconi's anaemia, therapy-related AML or extramedullary leukaemia without bone marrow involvement were excluded.

Diagnosis and therapy was centralized to the University Hospitals in the five countries and included 21 centres. Informed consent and ethical approval were obtained according to national regulations.

The diagnosis was established by analysing bone marrow aspirates, including standard morphology according to the French–American–British (FAB) classification (Bennett *et al*, 1985).

Central nervous system (CNS) involvement was diagnosed if more than 5 leucocytes/μl were identified in the cerebrospinal fluid (CSF) in combination with detectable leukaemic cells in the cytospin and/or with occurrence of neurological symptoms (e.g. cranial nerve palsy).

Complete remission (CR) was defined as a normocellular bone marrow (BM) with less than 5% blasts and haematological recovery (ANC > $1{\cdot}0 \times 10^9$/l, platelets > 80×10^9/l). BM aspiration was done at d 15–19 and then weekly until there was evidence of CR or persistent disease. Resistant disease was defined as residual leukaemia after three induction courses.

Cytogenetics. Bone marrow and/or peripheral blood samples were analysed using standard methods by different laboratories in the Nordic countries. In addition to the banded chromosome analysis, fluorescence *in situ* hybridization (FISH) as well as direct analyses for *MLL* rearrangement (Southern blot or FISH) were increasingly used to verify, or to describe more precisely, the changes found. The clonality criteria and the description of abnormalities have followed the recommendations of the International System for Human Cytogenetic Nomenclature (ISCN, 1995). The karyotypes of all patients entered on NOPHO-AML93 were reviewed by the NOPHO cytogenetic working group.

Treatment. Two or three induction courses were followed by four courses of high-dose cytarabine-based consolidation. The flow charts for the treatment of NOPHO-AML88 and NOPHO-AML93 are shown in Fig 1: **ATEDox** (cytarabine 200 mg/m^2 continuous infusion d 1–4, 6-Thioguanine 100 mg/m^2 b.i.d. orally d 1–4, etoposide 100 mg/m^2 continuous infusion d 1–4, doxorubicin 75 mg/m^2 4-h infusion d 5), **AM** (cytarabine 100 mg/m^2 continuous infusion d 1–5, mitoxantrone 10 mg/m^2 30-min infusion d 1–3), **HA$_1$M** (cytarabine 1 g/m^2 2-h infusion b.i.d. d 1–3, mitoxantrone 10 mg/m^2 30-min infusion d 3–5), **HA$_2$E** (cytarabine 2 g/m^2 2-h infusion b.i.d. d 1–3, etoposide 100 mg/m^2 60-min infusion d 2–5), **HA3** (cytarabine 3 g/m^2 2-h infusion b.i.d. d 1–3). For children under 2 years of age, chemotherapy was calculated per kilogram bodyweight with one square metre equalling 30 kg. Intrathecal methotrexate was given on the first day of each course at age-adjusted doses: below 1 year, 6 mg; 1 year old, 8 mg; 2 years old, 10 mg; 3 years and older, 12 mg.

In NOPHO-AML88, all patients were treated uniformly with three induction courses (ATEDox–AM–ATEDox). The interval between start of the induction courses 1 and 2 was

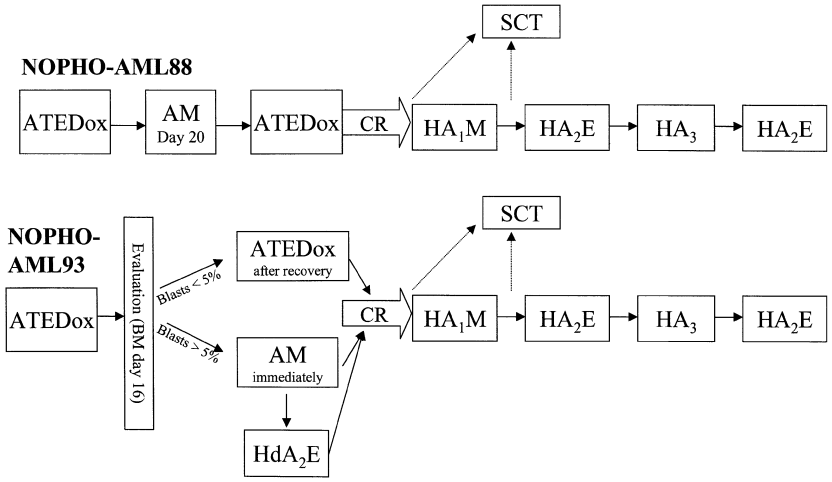

Fig 1. Flow chart of the treatment schedule for the NOPHO-AML88 and NOPHO-AML93 protocols. Details of the chemotherapy regimens are given in the text.

intended to be only 16 d, but was dependent on the condition of the patient.

In NOPHO-AML93, the courses were unchanged but patients were stratified to different induction treatment arms according to response after the first course of ATEDox. Good responders (defined as those with no evidence of leukaemia after 2–3 weeks) received a second course of ATEDox after haematological recovery (ANC > $1{\cdot}0 \times 10^9$/l, platelets > 80×10^9/l). Poor responders received AM and, if leukaemia still persisted, the child received HA_2E.

Consolidation consisted of four courses of high-dose cytarabine-based therapy (HA_1M–HA_2E–HA3–HA_2E) in both NOPHO-AML88 and NOPHO-AML93. The total number of courses was reduced from seven in NOPHO-AML88 to six in NOPHO-AML93, with the exception of the few patients who failed to obtain remission after two induction courses.

Regardless of the induction therapy, patients were treated with the same four consolidation courses as in NOPHO-AML88. Allogeneic stem cell transplantation (SCT) was recommended to all patients in first remission with a human leucocyte antigen (HLA)-matched family donor (MFD). Matched unrelated donor (MUD) transplantation was performed in a few patients at the discretion of the treating physicians.

Treatment with autologous SCT (ASCT) was optional during NOPHO-AML88 and the first part of NOPHO-AML93, and was performed on a non-randomized basis decided by the treating centres. In the present analysis, ASCT has been included in the chemotherapy only group.

Statistical analysis. The STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES (SPSS) software was used in the statistical analysis. The probability of EFS was calculated using the Kaplan–Meier method, and the different subgroups were compared using the log-rank test. The significance limit for *P*-values was set to 0·05 in all tests. In the analysis of EFS, events included induction failures (early death, death in aplasia and resistant disease), death in remission, relapse and second malignancy. Children who did not achieve remission were excluded from the analyses of disease-free survival (DFS). The cumulative incidences of relapse and death in remission were calculated according to the 'one minus survival' method (Mantel, 1966). Stepwise multiregression analysis, according to Cox, was used to identify prognostic risk factors. Differences between mean of days between courses and protocols were tested by using the analysis of variance (ANOVA) (Mantel, 1966). All patients were followed for adverse events until 1 January 2002. No patients were lost to follow-up.

RESULTS

Patients

NOPHO-AML88 and -AML93 included a total of 323 patients: the clinical characteristics are presented in Table I. There was no significant difference in the patient characteristics between the two populations.

The median age at presentation was 6·0 years, 12% were infants less than 1 year of age and 32% were 10-years old or more at diagnosis. CNS involvement was diagnosed in 6%. The median white blood cell count (WBC) at diagnosis was $14{\cdot}2 \times 10^9$/l, and 25% of the children had WBC $\geq 50 \times 10^9$/l.

Overall outcome

Table II and Figs 2 and 3 present the main results of the two protocols. The remission rate was 87% and 91% in NOPHO-AML88 and NOPHO-AML93 respectively. The EFS and overall survival (OS) at 7 years increased from 41% ± 5% and 47% ± 5% for NOPHO-AML88 to 49% ± 4% and 64% ± 4% for NOPHO-AML93 ($P = 0{\cdot}06$ and $P < 0{\cdot}01$).

Response to induction treatment

CR was obtained in 87% of the 104 children treated according to NOPHO-AML88, 10 died in aplasia and four had resistant disease (Table II). Owing to the intensity of the protocol, good responders could not be identified.

CR was achieved in 143 (65%) of the 219 patients in NOPHO-AML93 after the first induction course. Four children died before achieving remission (three early deaths, one death in aplasia). The 72 poor responders were treated with AM. Forty-three patients (61%) achieved remission after AM, two died in aplasia, four non-responders were taken off protocol, while 23 patients still not in remission were treated with HA_2E. Fourteen of these patients (63%) achieved remission while nine patients had resistant disease and were classified as induction failures. The resulting CR rate was 91% (Table II).

The number of days between the courses was analysed as a marker of dose intensity. The mean/median number of days between the start of the first and second induction course in NOPHO-AML88 was 22/21 d. The mean/median interval in NOPHO-AML93 between the start of the two ATEDox courses was 32/31 d, and between ATEDox and AM the interval was 33/27 d. The interval between the first two induction courses was significantly longer in NOPHO-AML93 than in NOPHO-AML88 ($P < 0{\cdot}01$). The number of days between subsequent courses did not differ between the protocols.

Post-remission therapy

SCT in first remission was performed in 18 patients (20%) treated on NOPHO-AML88 (16 MFD, two MUD) and in 53 (27%) treated on NOPHO-AML93 (46 MFD, seven MUD). ASCT was performed in 40 patients (NOPHO-AML88, $n = 24$; NOPHO-AML93, $n = 16$). DFS and OS for NOPHO-AML93 according to post-remission treatment are shown in Fig 4. The chemotherapy group included patients who received ASCT during consolidation. The SCT group consists of patients who were transplanted during consolidation with MFD or MUD. DFS was significantly superior in patients receiving SCT in first remission (64% *vs* 51%) (Fig 4A). However, the OS in patients who achieved remission was similar in those who received chemotherapy only or SCT in first remission (71% *vs* 69%) (Fig 4B). Subgroup analyses of OS in NOPHO-AML93 according to a poor or good response to the first induction course showed no difference between SCT and chemotherapy only (data not shown).

Table I. Characteristics of the patients at diagnosis with EFS and overall survival (OS) at 7 years from diagnosis.

	NOPHO-AML88				NOPHO-AML93				88 *vs* 93	
	n	%	EFS	OS	*n*	%	EFS	OS	p-EFS	p-OS
All patients	104	100	41 ± 5	47 ± 5	219	100	49 ± 4	64 ± 4	n.s.	< 0·01
Sex										
Male	52	50	31 ± 6	33 ± 6	109	50	57 ± 5	67 ± 5	< 0·01	< 0·01
Female	52	50	52 ± 7	62 ± 7	110	50	42 ± 5	62 ± 5	n.s.	n.s.
Age (years)										
< 1	15	14	53 ± 13	60 ± 13	25	11	44 ± 11	58 ± 11	n.s.	n.s.
1 – < 5	37	36	35 ± 8	43 ± 8	65	30	64 ± 6	76 ± 6	< 0·01	< 0·01
5 – < 10	18	17	50 ± 12	50 ± 12	59	27	46 ± 7	65 ± 7	n.s.	n.s.
≥ 10	34	33	38 ± 8	44 ± 9	70	32	41 ± 6	55 ± 6	n.s.	n.s.
WBC ($\times 10^9$/l)										
< 10	45	43	40 ± 7	44 ± 7	91	42	59 ± 5	73 ± 5	0·02	< 0·01
10 – < 50	36	35	39 ± 8	44 ± 8	71	32	46 ± 6	61 ± 6	n.s.	< 0·01
50 – < 100	6	6	33 ± 19	50 ± 20	32	15	44 ± 9	63 ± 9	n.s.	n.s.
≥ 100	17	16	53 ± 12	59 ± 12	25	11	39 ± 10	49 ± 11	n.s.	n.s.
CNS disease*										
Yes	4	4			14	6				
No	96	96			202	94				
FAB										
M0					14	6				
M1	23	22	36 ± 10	36 ± 10	32	15	37 ± 9	55 ± 9	n.s.	n.s.
M2	28	27	46 ± 9	50 ± 9	45	21	56 ± 8	67 ± 7	n.s.	n.s
M3	5	5			11	5				
M4	17	16	53 ± 12	71 ± 11	44	20	52 ± 8	70 ± 7	n.s.	n.s
M5	19	18	32 ± 11	37 ± 11	43	20	54 ± 8	70 ± 8	0·03	< 0·01
M6	1	1			5	2				
M7	3	3			13	6				
Unknown	9	9			12	5				

*Unknown in seven patients.

Owing to the small number of patients, EFS and OS were not calculated in patients with CNS involvement, and in some FAB subgroups. n.s., non-significant.

Table II. Main outcome of NOPHO-AML88 and NOPHO-AML93.

	NOPHO-AML88		NOPHO-AML93		
	n	%	*n*	%	*P*-value
All patients	104	100	219	100	
Early death	10	10	6	3	
Resistant disease	4	4	13	6	
Remission	90	87	200	91	
Relapse	38	36	79	36	
Death in CR	10	10	7	3	
CCR 1/2002	42	40	114	52	
EFS at 7 years		41 ± 5		49 ± 4	n.s.
DFS at 7 years		48 ± 5		54 ± 4	n.s.
OS at 7 years		47 ± 5		64 ± 4	< 0·01
Cumulated risk of					
Induction failure		14 ± 3		9 ± 2	n.s.
Death in CR1		12 ± 4		4 ± 2	0·02
Relapse		47 ± 6		44 ± 4	n.s.

n.s., non-significant; CCR 1/2002, continuous complete remission as of January 2002.

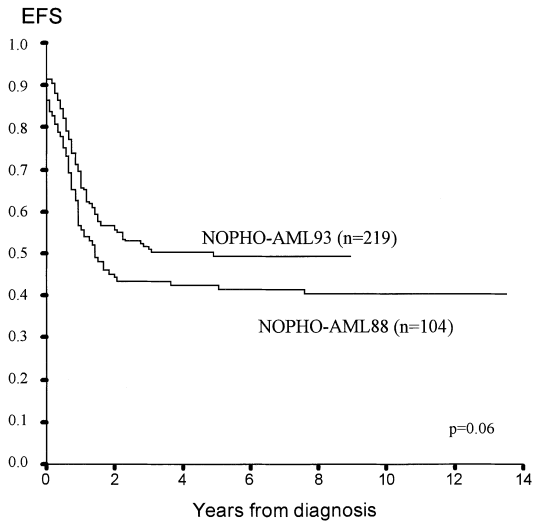

Fig 2. Probability of EFS in NOPHO-AML88 and NOPHO-AML93.

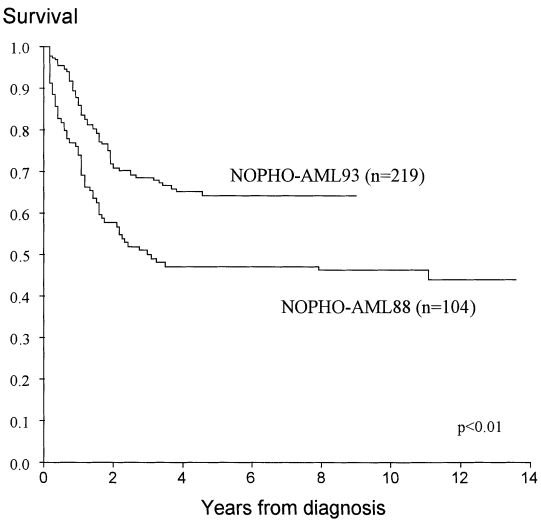

Fig 3. Probability of overall survival in NOPHO-AML88 and NOPHO-AML93.

Survival in NOPHO-AML93 was similar following SCT with an unrelated donor (71%) or related donor (70%). Survival following SCT showed no significant difference between NOPHO-AML88 (67%) and NOPHO-AML93 (71%).

Deaths in remission

Patients treated on NOPHO-AML88 had a high risk of toxic death in remission (10/90). Nine out of 10 deaths occurred within the first 7 months from diagnosis, and all deaths occurred among children who were treated with chemotherapy only. Compared with NOPHO-AML88, the toxic death rate in remission in NOPHO-AML93 decreased significantly to 3% ($P = 0{\cdot}008$) (Table II). Five of the seven deaths in first remission (CR1) occurred following SCT.

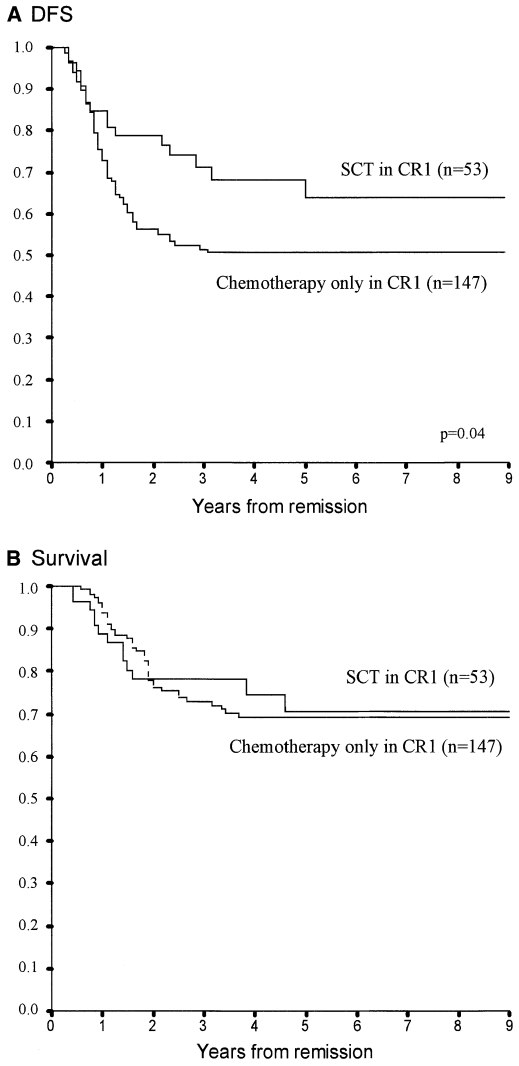

Fig 4. Probability of survival in NOPHO-AML93, according to postremission therapy in first CR: (A) disease-free survival (DFS) and (B) overall survival for patients achieving remission.

Relapses

The cumulative risk of relapse was 0·47 ± 0·06 in NOPHO-AML88 and 0·44 ± 0·04 in NOPHO-AML93. The relapses were predominantly located in the bone marrow (87%). Ten patients (8·5%) suffered from CNS ($n = 2$) or BM + CNS relapse ($n = 8$). Most relapses occurred within the first 3 years from diagnosis with a peak incidence between 6 and 18 months from diagnosis. There were three events in NOPHO-AML88 more than 3 years from diagnosis (two BM relapses after 44 months and 60 months, and one child who died 7 years after diagnosis from portal hypertension due to fungal infection during induction). In NOPHO-AML93, only two events occurred more than 3 years from diagnosis (BM relapses at 37 and 59 months).

Prognostic factors in NOPHO-AML93

Remission courses. The strongest prognostic factor in NOPHO-AML93 was the response to the first induction

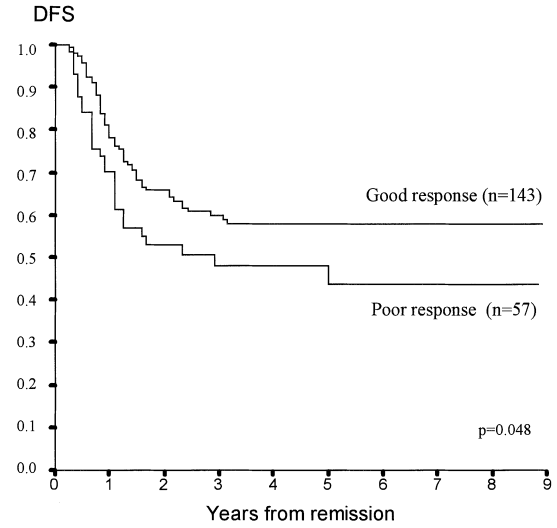

Fig 5. Probability of disease-free survival (DFS) in NOPHO-AML93, according to response to the first induction course.

course. Children who were good responders, achieving remission after first ATEDox (67% of the patients), had a significantly better outcome compared with poor responders (EFS 56% ± 4% *vs* 35% ± 6%, $P < 0{\cdot}01$) (Fig 5). The difference persisted even if induction failures were excluded from the analysis (good responders, DFS 58% ± 4%; poor responders, DFS 44% ± 5; $P = 0{\cdot}048$) (Fig 5).

WBC at diagnosis. WBC $\geq 50 \times 10^9$/l at diagnosis was associated with a higher rate of induction failures (19% *vs* 5%) with an EFS of 41% ± 7% compared with 52% ± 4% for children with WBC $< 50 \times 10^9$/l ($P < 0{\cdot}01$). Seven out of 10 children with resistant disease had WBC counts $\geq 50 \times 10^9$/l. WBC lost its prognostic impact for children achieving CR.

Age at diagnosis. Children aged 10 years or older ($n = 70$) had an inferior prognosis compared with younger children ($n = 149$) in the NOPHO-AML93 protocol (EFS 41% ± 6% *vs* 53% ± 4%, $P = 0{\cdot}04$). Eleven of the 70 children over 10 years of age experienced induction failure (16%), and 5/7 children who died during remission were children > 10 years of age at diagnosis (3/5 after SCT). The relapse rate was the same for the two age groups.

Sex. NOPHO-AML93 did not show any significant difference between boys and girls (EFS 57% ± 5% *vs* 42% ± 5%, $P = 0{\cdot}1$).

FAB classification. EFS varied between 35% and 75%, according to the FAB group. Several FAB subgroups were too small for reliable conclusions regarding the prognostic value. M1 did poorly in both NOPHO-AML88 and -AML93: in the -AML93 protocol, the difference was significant ($P = 0{\cdot}03$).

Chromosomal aberrations. Successful karyotype analysis was performed in 207/219 (95%) of the patients enrolled in the NOPHO-AML93 study. Clonal aberrations were found in 73% of patients (Table III). The largest specific subgroup was those with 11q23/*MLL* rearrangements ($n = 32$), the most common being t(9;11)(p22;q23) ($n = 16$). *MLL* rearrangements were identified by FISH or Southern blot in four patients with a G-banded karyotype without 11q23 involvement. Although the paucity of patients in each specific subgroup made outcome analysis uncertain, one subgroup differed significantly in survival, namely those with t(9;11), showing an EFS of 86% ± 9% compared with an EFS of 47% ± 4% in the remaining patients ($P < 0{\cdot}01$). Patients with t(8;21)(q22;q22) and chromosome 16 aberrations [inv(16)(p13q22) and t(16;16)(p13;q22)] tended to have a superior outcome. The combination of t(9;11), t(8;21) or chromosome 16 aberrations identified a favourable cytogenetic group ($n = 44$) with an EFS of 67% ± 7% compared with 44% ± 4% in patients without these aberrations ($P = 0{\cdot}02$). Those with hyperdiploidy (> 50 chromosomes, $n = 6$; EFS 33% ± 19%) and those with 11q23/*MLL* aberrations after exclusion of t(9;11) ($n = 16$; EFS 36% ± 12%) seemed to do worse but the differences were not statistically significant.

DISCUSSION

We have compared two consecutive protocols for the treatment of childhood AML and showed a significant improvement in overall outcome in the most recent one. The protocol NOPHO-AML88 was based upon the concept

Table III. Cytogenetic findings in NOPHO-AML93 with EFS and overall survival (OS) at 7 years from diagnosis.

	n	%	Events	EFS (%)	OS (%)
Diploid	55	27	30	42 ± 7	54 ± 7
t(8;21)(q22;q22)	18	9	8	56 ± 12	77 ± 10
t(9;11)(p22;q23)	16	8	2	86 ± 9	94 ± 6
11q23 aberrations other than t(9;11)	16	8	10	36 ± 12	44 ± 19
inv(16)/t(16;16)	10	5	4	60 ± 15	77 ± 15
t(15;17)(q22;q12)	8	4	4	47 ± 19	63 ± 23
> 50 chromosomes	6	3	4	33 ± 19	50 ± 20
Other abnormalities	78	38	39	48 ± 6	63 ± 6
Unknown	12		4	62 ± 15	83 ± 11

of increased dose intensity during induction in order to reduce the rate of resistant disease, which was considered too high in our previous less-intensive protocol (NOPHO-AML84) (Lie *et al*, 1996). The first two courses in NOPHO-AML88 were given close together as timed sequential therapy as investigated by the Children's Cancer Group (CCG) (Woods *et al*, 1996, 2001). Our results were not quite as expected. Although the proportion of resistant disease during induction decreased from 14% in NOPHO-AML84 to 4% in NOPHO-AML88, the number of children that died in aplasia increased from 7% to 10%. The number of children dying in CR was also too high (10%). The morbidity and mortality problems thus outweighed the possible benefit of the intensified therapy (Lie *et al*, 1996).

Our results are in some conflict with recent trends in the therapy of childhood AML. The concept of intensive-timing induction as practised by the CCG (Woods *et al*, 1996, 2001) produced superior results compared with more conventional approaches. The second course of induction in NOPHO-AML88 was often delayed and given at (median) d 21 in contrast to d 10 in CCG-2891 (Woods *et al*, 1996). The different timing may result in different recruitment of cells into S phase of the cell cycle, influencing both toxicity and remission. However, even in the best arm of the CCG study, the results were not superior to ours. Also the British and German childhood AML studies resulted in favourable outcome without such a timed-intensive induction therapy (Stevens *et al*, 1998; Creutzig *et al,* 2001).

The NOPHO-AML93 concept of allowing those 65% who entered remission after the first course of ATEDox to recover resulted in an increased interval between the first and the second induction course from 21 to 31 d. The median interval between ATEDox and AM was 27 d. According to the protocol, AM should be given if there was evidence of persistent leukaemia on d 16 following ATEDox, and the interval between ATEDox and AM was expected to be shorter. The main explanation for the lack of shortening of the interval was the fact that the bone marrow on d 16 often was too hypoplastic for evaluation, even in patients where repeated bone marrow examinations eventually showed persistent leukaemia.

The most important prognostic factor was the response to the first course of therapy. Children with persistent disease after the first course received a different and more toxic induction therapy, while those that responded were spared such intensification. The EFS of the good responders was significantly better than that of the poor responders (56% *vs* 35%). The prognostic importance of the response to the initial therapy has also been shown by other study groups (Creutzig *et al*, 1999; Wheatley *et al*, 1999; Wells *et al,* 2002).

The patients were genetically randomized to receive MFD. Patients receiving SCT had fewer relapses but, as a result of a high salvage rate in those relapsing following chemotherapy only, the overall survival between the two groups did not differ. This is in contrast to the results from other groups (Woods *et al,* 2001). The overall survival of 69% in NOPHO-AML93 in patients not transplanted in CR1 was comparable to the best results in patients receiving SCT (Woods *et al,* 2001). The comparable survival in the chemotherapy and SCT group in first CR may lead to more restrictive indications for SCT, reserving the procedure for poor-risk patients and relapsed patients (Burnett *et al,* 2002; Creutzig & Reinhardt, 2002).

The overall results of NOPHO-AML93 with an EFS of 49% and overall survival of 64% compare favourably with other AML studies. CCG-2891 showed DFS and overall survival of 55% and 63%, respectively, in the intensive-timing group (Woods *et al*, 1996). The Medical Research Council (MRC) AML10 and 12 studies produced an EFS and overall survival in children of 52% and 61% respectively (Webb *et al,* 2001). The MRC protocol included a high dose of anthracyclines that may cause concern for long-term side-effects. The Berlin–Franfurt–Munster (BFM) protocol (AML-BFM 93) achieved an EFS and overall survival of 51% and 60% respectively (Creutzig *et al,* 2001). The BFM protocol contains cranial irradiation that may cause long-term toxicity. The NOPHO protocol contains a moderate amount of anthracycline and a high cumulative dose of cytarabine. The courses of cytarabine were well tolerated. Although no systematic long-term evaluation has been performed, we are not aware of any cytarabine-associated long-term effects in our patients.

We previously found that girls did significantly better than boys (Lie *et al*, 1996). In NOPHO-AML93, this difference disappeared. This is surprising, as the difference in the first two protocols was quite large (EFS 43% *vs* 26%) and based upon data from 200 children. There was no difference between sex in CR rates or in the type of therapy received.

One characteristic of the Nordic AML material has been the high frequency of Down's syndrome (DS) (Lie *et al*, 1990). During the study period, myeloid leukaemia was diagnosed in 50 children with DS, corresponding to 13% of the patients with AML. Myeloid leukaemia in DS has, in recent years, been recognized as a unique biological entity (Hasle *et al,* 2003). The prognosis of myeloid leukaemia in DS is significantly better than AML in non-DS (Lie *et al*, 1996; Lange *et al*, 1998; Ravindranath & Taub, 1999) and, therefore, we excluded DS from the present material, which resulted in a slightly lower EFS than previously reported for NOPHO-AML88 (Lie *et al*, 1996). This must be considered when comparing our results with those of trials including DS patients in the overall results.

The frequency of the cytogenetic aberrations in this study are in accordance with other large series (Grimwade *et al*, 1998; Raimondi *et al*, 1999). The most frequent recurrent aberrations in our material were 11q23/*MLL* rearrangements. Survival analyses showed a significantly better survival in children with t(9;11) (EFS 86%), whereas other 11q23 translocations were associated with a poor outcome. Data from St Jude Children's Hospital showed similarly an improved survival in t(9;11) (Pui *et al,* 2000; Rubnitz *et al,* 2002). Data from adults showed a favourable outcome in t(9;11) compared with other 11q23 aberrations, especially in patients who received intensive postremission chemotherapy with high-dose cytarabine (Mrozek *et al*, 1997). The inclusion of etoposide (also used at St Jude) in the

induction courses, followed by several courses of high-dose cytarabine during consolidation, may be the main reason for the improved survival in t(9;11) patients. *In-vitro* sensitivity studies have shown an increased cytotoxicity of etoposide and cytarabine in patients with t(9;11) (Zwaan *et al,* 2002).

None of the other cytogenetic groups showed any statistically significant differences in survival. However, a very poor outcome was noted in the small group (4% of the patients) with a hyperdiploid karyotype (> 50 chromosomes); the same tendency has been found by others (Wells *et al,* 2002). The survival of patients with t(8;21) and inv(16)/t(16;16) showed a non-significant trend for a better outcome, in accordance with other studies (Grimwade *et al*, 1998), especially when treated with high-dose cytarabine (Bloomfield *et al*, 1998). Combining t(9;11), t(8;21) and chromosome 16 aberrations, we identified a group with significantly superior outcome.

Identification of specific AML subgroups based upon cytogenetics and *in-vivo* response may provide more biologically based stratification, helping to select the optimal therapy for children with AML.

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