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Outcome after intensive reinduction therapy and allogeneic stem cell transplant in paediatric relapsed acute myeloid leukaemia

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Summary

Given that 30–40% of children with acute myeloid leukaemia (AML) relapse after primary therapy it is important to define prognostic factors and identify optimal therapy. From 1993 to 2012, 543 children from the Nordic countries were treated according to two consecutive protocols: 208 children relapsed. The influence of disease characteristics, first line treatment, relapse therapy and duration of first remission on outcome was analysed. Second complete remission (CR2) was achieved in 146 (70%) patients. Estimated 5-year overall survival (OS_{5y}) was 39 ± 4% for the whole group and 43 ± 4% for the 190 patients given re-induction therapy, of whom 76% received regimens that included fludarabine, cytarabine (FLA) ± anthracyclines, 18% received Nordic Society for Paediatric Haematology and Oncology (NOPHO) upfront blocks and 5% received other regimens. Late relapse ≥1 year from diagnosis, no allogeneic stem cell transplantation (SCT) in first remission and core binding factor AML were independent favourable prognostic factors for survival. For the 128 children (124 in CR2) that received SCT as consolidation therapy after relapse, OS_{5y} was 61 ± 5%. Four of 19 children (21%) survived without receiving SCT as part of relapse therapy. Our data show that intensive re-induction followed by SCT can give cure rates of 40% in children with relapsed AML.

Keywords: acute myeloid leukaemia, relapsed, childhood, allogenic stem cell transplant, survival.

In the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) children with *de novo* acute myeloid leukaemia (AML) have been treated according to cooperative NOPHO (Nordic Society for Paediatric Haematology and Oncology) AML trials since 1984. The 5-year event-free survival (EFS) and overall survival (OS) has improved considerably, from 29% to 38%, respectively on the NOPHO-AML-84 trial, to 47% and 70% on the NOPHO-AML 2004 trial (Lie *et al*, 2005; Abrahamsson *et al*, 2011). Despite the improved outcome the cumulative risk of relapse after primary therapy continues to

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be around 30–40% in both NOPHO and other contemporary treatment protocols (Lie *et al*, 2005; Zwaan *et al*, 2015).

Paediatric AML study groups have attempted to further reduce the frequency of relapse mainly by intensification of induction therapy but also by stratifying treatment according to early treatment response and cytogenetic aberrations (von Neuhoff *et al*, 2010; Rubnitz *et al*, 2010; Abrahamsson *et al*, 2011). Many studies have demonstrated that allogeneic stem cell transplantation (SCT) in first complete remission (CR1) reduces the relapse rate with an increase in EFS, however without significant effect on OS (Niewerth *et al*, 2010). This has been attributed to higher treatment-related mortality in patients treated with SCT, combined with a higher salvage rate in patients treated with chemotherapy only (Abrahamsson *et al*, 2007). Still, it remains to be proven if SCT in CR1 increases survival in subgroups of patients with defined poor risk features, such as detectable minimal residual disease after induction or presence of poor risk cytogenetic aberrations (Pigazzi *et al*, 2015). At present, clear evidence-based indications are lacking and the use of SCT in first remission varies considerably between different study groups (Hasle, 2014).

The most successful paediatric AML trials have achieved a 5-year EFS of 60–65% (Tsukimoto *et al*, 2009; Imamura *et al*, 2012). However, most recent clinical AML trials also show an OS that is in the order of 10–15% higher than EFS (Tsukimoto *et al*, 2009; Rubnitz *et al*, 2010; Creutzig *et al*, 2013). Thus, the increase in OS in paediatric AML partly depends on improved treatment of relapse with a substantial proportion of children being cured following relapse.

Previously, survival rates after AML relapse were very low and many children only received palliative therapy. However, more recent studies have shown that intensive re-induction therapy followed by allogeneic SCT can lead to cure in 30–40% of cases (Abrahamsson *et al*, 2007; Kaspers *et al*, 2013).

In an attempt to further characterize prognostic factors and important treatment elements in relapsed paediatric AML, we investigated children with AML who relapsed after therapy according to two consecutive NOPHO study protocols. The studies were population-based and included 543 children with *de novo* AML treated from 1993 to 2012. Two hundred and eight children relapsed: we analysed the outcome after relapse in relation to patient and disease characteristics, primary treatment and relapse therapy.

Methods

Between January 1993 and December 2012, 543 children aged 0–18 years from the Nordic countries with *de novo* AML were registered and treated according to the two consecutive study protocols NOPHO-AML 93 and NOPHO-AML 2004. Patients with Down syndrome and acute promyelocytic leukaemia were not included. The study includes all 208 children who had a relapse before the 1st April 2014.

Primary treatment

Figure 1 outlines the therapy used in the trials for *de novo* AML.

The NOPHO-AML 93 study was the first protocol in the Nordic countries with a response-guided approach (Abrahamsson *et al*, 2011). Patients with good response to the first course waited until haematological recovery before receiving a second course of ATEDox (cytarabine, etoposide, thioguanine, doxorubicin) while patients with poor response immediately proceeded to a different second course, AM (cytarabine, mitoxantrone). Consolidation therapy consisted of four courses. All patients with a human leucocyte antigen (HLA) identical sibling donor were recommended for SCT in CR1. Autologous bone marrow transplantation (ABMT) was initially recommended for children who did not achieve remission after course one and lacked a sibling donor but compliance was very low and few patients actually received ABMT. NOPHO-AML 93 was a very effective protocol with an EFS of 50% and OS of 66% at 5 years (Lie *et al*, 2005).

NOPHO-AML 2004 retained the same response-guided approach but all patients received AIET (cytarabine, etoposide, 6-thioguanine, idarubicin) as first course and AM as second. Consolidation therapy was identical to the NOPHO-AML 93 study with the exception that patients with standard risk after consolidation were randomised to either no further therapy or two post-consolidation courses of single-drug gemtuzumab (5 mg/m^2) (Hasle *et al*, 2012). In NOPHO-AML 2004, SCT in CR1 was recommended to all high-risk patients with an HLA-identical donor (sibling or unrelated). High risk patients included those with poor response, defined as more than 15% blasts on day 15 after the first induction course or no complete remission after two induction courses. Patients with *KMT2A* [11q23] rearrangements other than *KMT2A/MLLT3* [t(9:11)(p22;q23)] were also classified as high risk until 2010 when the protocol was amended, including *FLT3*-internal tandem duplication (ITD) mutation as a high-risk criterion instead. Compared to NOPHO-AML 93 the OS_{5y} improved to 70% while EFS_{5y} was 47% (Abrahamsson *et al*, 2011).

Relapse treatment

In the NOPHO-AML 93 era, treatment of relapsed AML in children was heterogeneous because the protocol did not offer any guidelines. Patients received elements from upfront treatments protocols, alternative regimens or palliative treatment.

The NOPHO-AML 2004 treatment protocol recommended that children with relapsed AML should be treated according to FLAG-based [fludarabine, cytarabine, granulocyte colony-stimulating factor (G-CSF)] regimens in line with the relapsed trial AML 2001/01 (Kaspers *et al*, 2013). Twenty-two patients in the present study were included in AML 2001/01, in which re-induction consisted of two

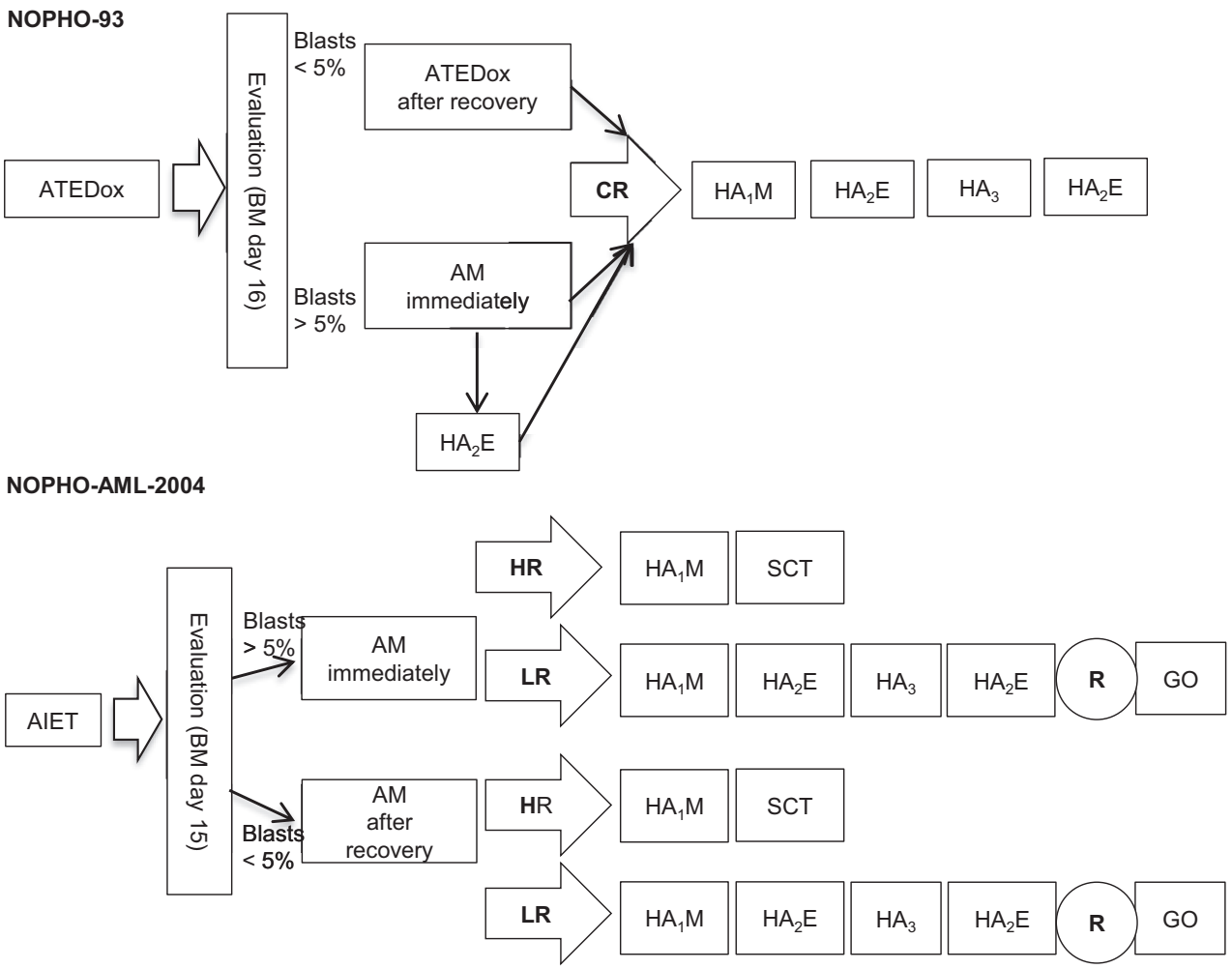

Fig 1. Outline of Nordic Society for Paediatric Haematology and Oncology 93 (NOPHO) and NOPHO-AML 2004 protocols. ATEDox – cytarabine 200 mg/m^2 and etoposide 100 mg/m^2 c.i. days 1–4, thioguanine 100 mg/m^2 orally every 12 h, days 1–4, doxorubicin 75 mg/m^2 per day, AM – cytarabine 100 mg/m^2 c.i. days 1–5, mitoxantrone 10 mg/m^2 days 3–5. HA_1M – cytarabine 1 g/m^2 × 2 days 1–3, mitoxantrone 10 mg/m^2 days 3–5. HA_2E – cytarabine 2 g/m^2 × 2 days 1–3, etoposide 100 mg/m^2 days 2–5. HA_3 – cytarabine 3 g/m^2 × 2 days 1–3. AIET – cytarabine 200 mg/m^2 and etoposide 100 mg/m^2 c.i. days 1–4, thioguanine 100 mg/m^2 orally every 12 h, days 1–4, idarubicin 12 mg/m^2 days 2–6. GO – gemtuzumab 5 mg/m^2 days 1–21. Before each course one intrathecal injection of methotrexate in age-adapted dose was given. BM, bone marrow; HR, high risk; LR, low risk; R, randomization; SCT, stem cell transplantation.

courses of FLAG, including a randomisation with or without the addition of liposomal daunorubicin in the first course. Based on the results from the AML 2001/01 trial a new recommendation was issued in November 2009 (Kaspers *et al*, 2013) using FLAG and liposomal daunorubicin as the first course followed by FLAG as the second course, aiming for consolidation with SCT if possible. The inclusion of G-CSF in the FLAG course was gradually abandoned from 2010.

Definitions

Bone marrow relapse was defined as the presence of ≥5% leukaemic cells on examination of bone marrow morphology and central nervous system (CNS) relapse as ≥5 leukaemic cells per μl in the cerebrospinal fluid. Complete remission was defined as <5% leukaemic cells on morphological examination of a non-hypoplastic bone marrow with regeneration of peripheral blood cells and no evidence of extra medullary disease.

Early relapse was defined as relapse less than 1 year from primary diagnosis and late relapse as ≥1 year from diagnosis.

Statistical methods

IBM SPSS Statistics for MAC, Version 21 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. Differences in proportions were assessed with Fisher's exact test for 2 × 2 contingency tables and Pearson's chi-squared for higher order tables. Median values were compared using Mann-Whitney *U*-test. The Kaplan–Meier method was used

to estimate survival rates and differences between factors were tested with the log-rank test. Survival was calculated from the date of diagnosis of relapse to death of any cause. For EFS analyses, resistant disease following relapse, second relapse, second malignancy or death not caused by progressive disease were considered as events. All living patients were censored at time of last follow up but not later than 1 April 2014. To evaluate the impact on survival of both categorical and continuous variables Cox regression was used. All *P*-values were two sided, and considered significant when <0·05. Estimates of survival were given as % probability of 5 years survival ± standard error (SE).

Results

Patient characteristics

The cumulative incidence of relapse at 5 years was 44% for both NOPHO-AML 93 and NOPHO-AML 2004. In all, 208 of 543 children experienced relapse: 112 of the 282 (40%) patients treated on NOPHO-AML 93 and 96 of 261 patients (37%) on NOPHO-AML 2004 relapsed. Patient characteristics are shown in Tables I and II. Of the 208 patients that relapsed, 110 (53%) had early relapse and 98 (47%) had late relapse. The median time from diagnosis to relapse was 12 months.

One hundred and eighty-three (88%) patients, had isolated bone marrow relapse and 12 (6%) had bone marrow combined with CNS relapse. Six patients had bone marrow relapse combined with other sites (two testis, two cutaneous, one lung and one mediastinal). Three patients had isolated CNS relapse and four patients had isolated extramedullary relapse.

For the relapses, patient characteristics were generally similar between the study protocols. However, some cytogenetic groups were differently distributed (Table II). *RUNX1/RUNX1T1* [t(8;21)(q22;q22)] was less common in relapsed patients in NOPHO-AML 93 (7/108 vs. 19/96; $P = 0{\cdot}004$). Also, the number of patients with *KMT2A/MLLT3* [t(9:11)(p22;q23)] was lower in NOPHO-AML 93 (4/108 vs. 13/96; $P = 0{\cdot}011$). This was caused by an increased relapse rate in NOPHO-AML 2004 in these cytogenetic subgroups.

Remission induction

Figure 2 shows an overview of the therapy for all patients. There was no significant difference in CR2 rates between NOPHO-AML 93 [74/112 (66%)] and NOPHO-AML 2004 [72/96 (75%); $P = 0{\cdot}16$]. There was a trend for a higher CR2 rate in patients receiving chemotherapy only in primary treatment [130/178 (73%)] compared to those treated with SCT [13/24 (54%); $P = 0{\cdot}056$]. Patients with early relapse had a lower CR2 rate [60/110 (54%)] than those with late relapse [86/98 (88%); $P < 0{\cdot}0001$].

Patients not receiving re-induction treatment

Seventeen of the 207 patients with treatment data received palliative therapy. Only four of these patients were treated on NOPHO-AML 2004. 88% (15/17) had early relapse compared to 49% (93/188) treated with re-induction therapy ($P = 0{\cdot}01$). Also, a higher proportion of the patients receiving palliative therapy (9/17) were treated with SCT in CR1 compared to 15/188 receiving re-induction therapy ($P < 0{\cdot}0001$). Three of the patients given palliative therapy entered CR2, but they all died with a median survival of 19 weeks.

Patients receiving re-induction treatment

In all, 92% (190/207) received re-induction therapy. The proportion of patients given re-induction was higher in NOPHO-AML 2004 [92/96 (96%)] than in NOPHO-AML 93 [92/111 (88%), $P = 0{\cdot}049$]. Of patients who received re-induction treatment, 75% achieved CR2. Table III shows the frequency and remission rate for the different re-induction regimens. Although the CR2 rate was similar in the two protocols, it is notable that significantly more patients received FLA-based re-induction and fewer NOPHO upfront protocols as re-induction therapy in NOPHO-AML 2004. From 2002, a total of 22 patients from Denmark, Norway and Finland were included in the AML2001/01 trial (Kaspers *et al*, 2013). Of these 22 patients, 13 were treated on NOPHO-AML 93 and nine on NOPHO-AML 2004.

The incidence of both early and late relapse was about 50% (94/190 vs. 96/190). The CR2 rate for patients with late relapse was significantly higher than for patients with early relapse [90% (86/96) vs. 61% (57/94); P=<0·0001]. CR2 rates were similar in patients treated with SCT (12/15), ABMT (2/4) and chemotherapy (129/171) in CR1 ($P = 0{\cdot}460$). All patients with *CBFB/MYH11 [inv(16)(p13q22)]* achieved CR2 (11/11), 89% (23/26) of patients with *RUNX1/RUNX1T1*, 80% (12/15) with *KMT2A/MLLT3* and 75% (15/20) with other *KMT2A* rearrangement. The difference between the groups was not significant. Of the 18 patients with *FLT3*-ITD aberration, 12 (67%) obtained CR2. Neither gender, age, French-American-British classification (FAB) type nor protocol at initial diagnosis correlated with second remission rate.

Survival

Overall, the estimated probability for 5-years survival was 39 ± 4%. There was no significant difference in OS_{5y} for patients treated on NOPHO-AML 2004, 44 ± 5% compared to NOPHO-AML 93, 36 ± 5% ($P = 0{\cdot}471$), (Fig 3A). For the patients given re-induction therapy OS_{5y} was 43 ± 4%.

Consolidation therapy in CR2 and survival

No patient who did not receive re-induction therapy survived. For patients achieving CR2, OS_{5y} was 54 ± 4%. In the

Table I. Presenting features and outcome in all patients ($N = 208$) with AML relapse.

	N	%	5-year OS (%)	*P*-value
All patients	208	100	39 ± 4	
Protocol				
NOPHO-AML 93	112	54	36 ± 5	
NOPHO-AML 2004	96	46	44 ± 5	0·471
Gender				
Male	104	50	39 ± 5	
Female	104	50	37 ± 5	0·465
Age (years)				
<2 years	45	22	35 ± 7	
2–9 years	94	45	42 ± 5	0·369
≥10 years	69	33	34 ± 6	
WBC at diagnosis				
$<100 \times 10^9$/l	186	89	40 ± 4	
$\geq 100 \times 10^9$/l	22	11	29 ± 10	0·181
FAB type				
M0	13	6	12 ± 10	
M1	33	16	30 ± 10	
M2	59	28	49 ± 7	
M4	36	17	54 ± 9	0·007
M5	36	17	27 ± 8	
M6	3	1	0	
M7	20	10	38 ± 11	
No data	8	4	40 ± 20	
Genetic aberration‡				
RUNX1/RUNX1T1	26	13*	57 ± 10	
CBFB/MYH11	11	5*	60 ± 16	0·083
KMT2A/MLLT3	17	8*	43 ± 13	
Other *KMT2A* rearrangement	22	11*	25 ± 10	
FLT3-ITD	18	14†	21 ± 10	
Time to relapse				
<1 year	110	53	24 ± 4	
≥1 year	98	47	55 ± 6	<0·0001
Consolidation in CR1				
Chemotherapy	184	88	42 ± 4	
SCT	24	12	13 ± 7	0·009
Relapse site				
Isolated BM	183	88	41 ± 4	
BM and CNS	12	6	36 ± 15	
CNS	3	1	0	<0·0001
BM and other sites	6	3	0	
Extra-medullary	4	2	25 ± 22	
Second complete remission				
Yes	146	70	54 ± 4	
No	62	30	3 ± 2	<0·0001

AML, acute myeloid leukaemia; BM, bone marrow; CNS, central nervous system; CR1, first complete remission; CR2, second complete remission;FAB, French-American-British classification; ITD, internal tandem duplication; NOPHO, Nordic Society for Paediatric Haematology and Oncology; OS, overall survival; SCT, stem cell transplant; WBC, white blood cell count.
*Percentage related to $n = 204$ patients with data on cytogenetic (missing data $n = 4$).
†Percentage related to $n = 124$ patients with data on *FLT3*-ITD (missing data $n = 84$).
‡Genetic aberrations were determined at the time of initial diagnosis.

61 patients not achieving CR2, only the two patients who were given SCT as initial relapse therapy survived.

Five of the 146 patients in CR2 died within 3 months from relapse. Four of these experienced treatment-related death whereas one died following a second relapse. Of the 141 patients remaining in CR2 and alive at 3 months from relapse date, 123 received consolidation therapy with SCT and 16 with chemotherapy. Two patients had only palliative

Table II. Presenting features according to primary treatment protocol.

	NOPHO-AML 93 n = 112 N (%)	NOPHO-AML 2004 n = 96 N (%)
Gender		
Male	48 (43)	56 (58)
Female	64 (57)	40 (42)
Age (years)		
<2 years	27 (24)	18 (19)
2–9 years	52 (46)	42 (44)
≥10 years	33 (30)	36 (38)
WBC at diagnosis		
$<100 \times 10^9$/l	101 (90)	85 (89)
$\geq 100 \times 10^9$/l	11 (10)	11 (11)
CNS involvement at diagnosis		
Yes	7 (6)	8 (8)
No	104 (93)	88 (92)
No data	1 (1)	0
FAB type		
M0	9 (8)	4 (4)
M1	17 (15)	16 (17)
M2	26 (23)	33 (34)
M4	24 (21)	12 (13)
M5	17 (15)	19 (20)
M6	1 (1)	2 (2)
M7	13 (12)	7 (7)
No data	5 (5)	3 (3)
Genetic aberration		
RUNX1/RUNX1T1	7/108 (7)	19/96 (20)
CBFB/MYH11	5/108 (5)	6/96 (6)
KMT2A/MLLT3	4/108 (4)	13/96 (14)
Other *KMT2A*	15/108 (14)	7/96 (7)
FLT3-ITD	7/43 (16)	11/81 (14)
Time to relapse		
<1 year	57 (51)	53 (55)
≥1 year	55 (49)	43 (45)
Consolidation in CR1		
Chemotherapy	94 (84)	90 (94)
SCT	18 (16)	6 (6)
Relapse site		
Isolated BM	98 (88)	85 (89)
BM and CNS	8 (7)	4 (4)
CNS	1 (1)	2 (2)
BM and other sites	1 (1)	5 (5)
Extra-medullary	4 (4)	0
Second complete remission		
Yes	74 (66)	72 (75)
No	38 (34)	24 (25)
Alive/dead		
Alive	38 (34)	46 (48)
Dead	74 (66)	50 (52)
Events		
No event	34 (30)	42 (44)
RD	37 (33)	23 (24)
TRM	17 (15)	8 (8)
Second relapse	24 (21)	23 (24)

AML, acute myeloid leukaemia; BM, bone marrow; CNS, central nervous system; CR1, first complete remission; CR2, second complete remission;FAB, French-American-British classification; ITD, internal tandem duplication; NOPHO, Nordic Society for Paediatric Haematology and Oncology; RD, resistant disease; SCT, stem cell transplant; TRM, treatment related mortality; WBC, white blood cell count.

therapy. The median time to SCT was 103 days from date of relapse. Of the 18 patients alive in second remission after 3 months but not given SCT in CR2, 12 were treated with SCT in first remission.

There was an increase in the number of patients receiving SCT as part of relapse therapy on NOPHO-AML 2004 compared to NOPHO-AML 93 [76% (70/92) vs. 59% (58/98); $P = 0{\cdot}013$]. Patients receiving SCT as consolidation therapy after relapse (124 in CR2 and 4 not in CR2) had an estimated 5-year probability of survival of 61 ± 5%. There was no significant difference in survival between patients treated on the two protocols (64 ± 6% for NOPHO-AML 93 vs. 60 ± 6% for NOPHO-AML 2004). There was no significant difference in survival between children with a matched family donor (MFD; $n = 39$, OS_{5y} 65 ± 9%), matched (9/10 or 10/10 alleles) unrelated donor, (MUD; $n = 84$, OS_{5y} 57 ± 6%) or mismatched donor ($n = 5$, OS_{5y} 53 ± 25%). Treatment-related mortality for SCT with MFD and MUD was similar, [13% (5/39) vs. 17% (14/84)].

There was a large difference in survival between the children that received SCT and those that received chemotherapy only in CR2 (OS_{5y} 61 ± 5% vs. 18 ± 8%, $P < 0{\cdot}001$).

Factors associated with survival

Time from diagnosis to relapse. In univariate analyses, OS_{5y} was 24 ± 4% for early and 55 ± 6% for late relapse, ($P < 0{\cdot}0001$), (Fig 3B). Cox regression showed that time to relapse from diagnosis was the strongest independent predictor of outcome. (Table IV).

Consolidation in first remission. Survival was significantly lower in children who underwent SCT in CR1. Univariate analyses showed an estimated probability of 5-year survival of 13 ± 7% for those that had SCT in CR1 vs. 42 ± 4% for those treated with chemotherapy, ($P = 0{\cdot}009$), (Fig 3C). None of the six children who underwent ABMT in CR1 survived. Cox regression analyses confirmed the inferior outcome for children that were treated with SCT in CR1 ($P = 0{\cdot}001$; Table IV).

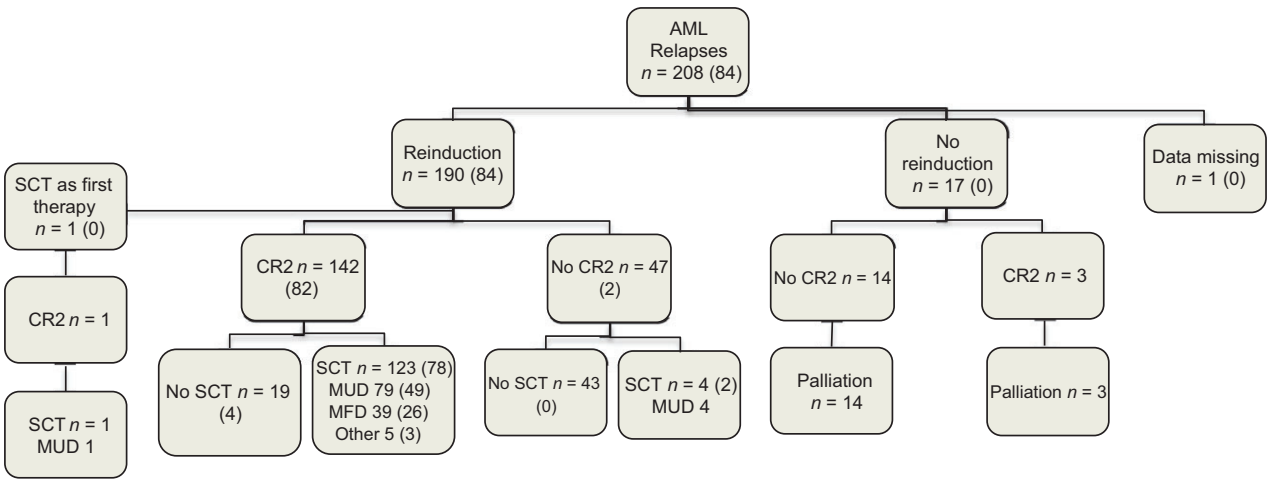

Fig 2. Flow diagram detailing the data on re-induction therapy, remission status and consolidation therapy in 208 patients with relapsed acute myeloid leukaemia. The numbers in parenthesis show the number of survivors within each group. AML, acute myeloid leukaemia; CR2, second complete remission; MFD, matched family donor; MUD, matched unrelated donor; SCT, stem cell transplantation.

Of the children treated on NOPHO AML-2004 protocol, 45 were randomised to either two post-consolidation courses of single-drug gemtuzumab (5 mg/m^2) or to no further therapy in CR1. There was no difference in survival between children that received gemtuzumab (n = 19, 47 ± 11%) and children that did not (n = 26, 61 ± 10%), (P = 0·547).

Core binding factor (CBF)-AML. In univariate analyses, the prognosis was significantly better for patients with CBF-AML (n = 37, 58 ± 8% vs. n = 167, 34 ± 4%), (P = 0·008), (Fig 3D). Cox regression analyses also showed that CBF-AML was a favourable prognostic independent predictor of outcome, (Table IV).

The following factors were included when performing the Cox regression analyses: time to relapse from diagnosis, age, gender, primary treatment protocol, consolidation therapy in CR1 and CBF-AML.

In total, 124 children died: 60 from resistant disease, 39 from second relapse and 25 from treatment-related causes. In the children with treatment-related death, 20 died from transplant-related complications. For the remaining five, data was missing in two and three died from sepsis, typhlitis and long-term aplasia, respectively. There was no significant difference in treatment-related death between NOPHO-AML 93 and NOPHO-AML 2004 [15% (17/25) vs. 8% (8/25); P = 0·130].

Eight children, of whom only three received reinduction therapy, suffered an early death, defined as death within 42 days from relapse.

Discussion

We demonstrate, in an unselected population-based cohort of children with relapsed AML, that almost 40% survived. There was no obvious difference in outcome between the NOPHO-AML 93 and NOPHO-AML 2004 trial.

In this cohort, 92% were given re-induction therapy and the 5-year OS in our 208 patients was 39%. In a randomised study comparing re-induction with FLAG and FLAG + liposomal daunorubicin, the International Berlin-Frankfurt-Münster (I-BFM) group, showed a 4-year OS of 38% in 394 patients (Kaspers *et al*, 2013). However, this study was not population-based and all patients received re-induction therapy. The OS_{5y} in our study, when excluding patients only receiving palliation, was 43%. In a paediatric study, the French Leucémie Aiquë Myéloïde Enfant (LAME) group treated 106 patients with the LAME 89/91protocol, 91% of whom received re-induction therapy, and demonstrated a 5-year OS of 33% (Aladjidi *et al*, 2003). In an older study from the UK AML 10 trial, 71% received re-induction therapy and a lower 3-year survival of only 24% was reported in a group of 125 patients (Webb *et al*, 1999).

Survival in relapsed patients from NOPHO-AML 2004 was 44% compared to 36% in NOPHO-AML 93. This was not statistically significant but the numbers in our cohorts were too low to allow detection of a 10% difference. Thus, the higher number in 2004 could indicate an improved outcome. On the other hand, this in turn might not depend on an improvement in therapy but could reflect that a higher fraction received re-induction therapy in NOPHO-AML 2004 and that a larger proportion of relapses in NOPHO-AML 2004 had favourable genetics.

The remission rate after relapse was high in our cohort: 70% of patients entered CR2, as compared to 64% of the children in the randomized I-BFM trial (Kaspers *et al*, 2013). The I-BFM trial achieved a higher CR rate with the addition of liposomal daunorubicin (L-DNR), 69% vs. 59% (Kaspers *et al*, 2013); however, the authors found no difference in OS between children receiving FLAG with L-DNR or FLAG without L-DNR. More patients treated on NOPHO-AML 2004 had FLA/FLAG or FLA+/FLAG+ than patients treated on NOPHO-AML 93, but there was no significant difference

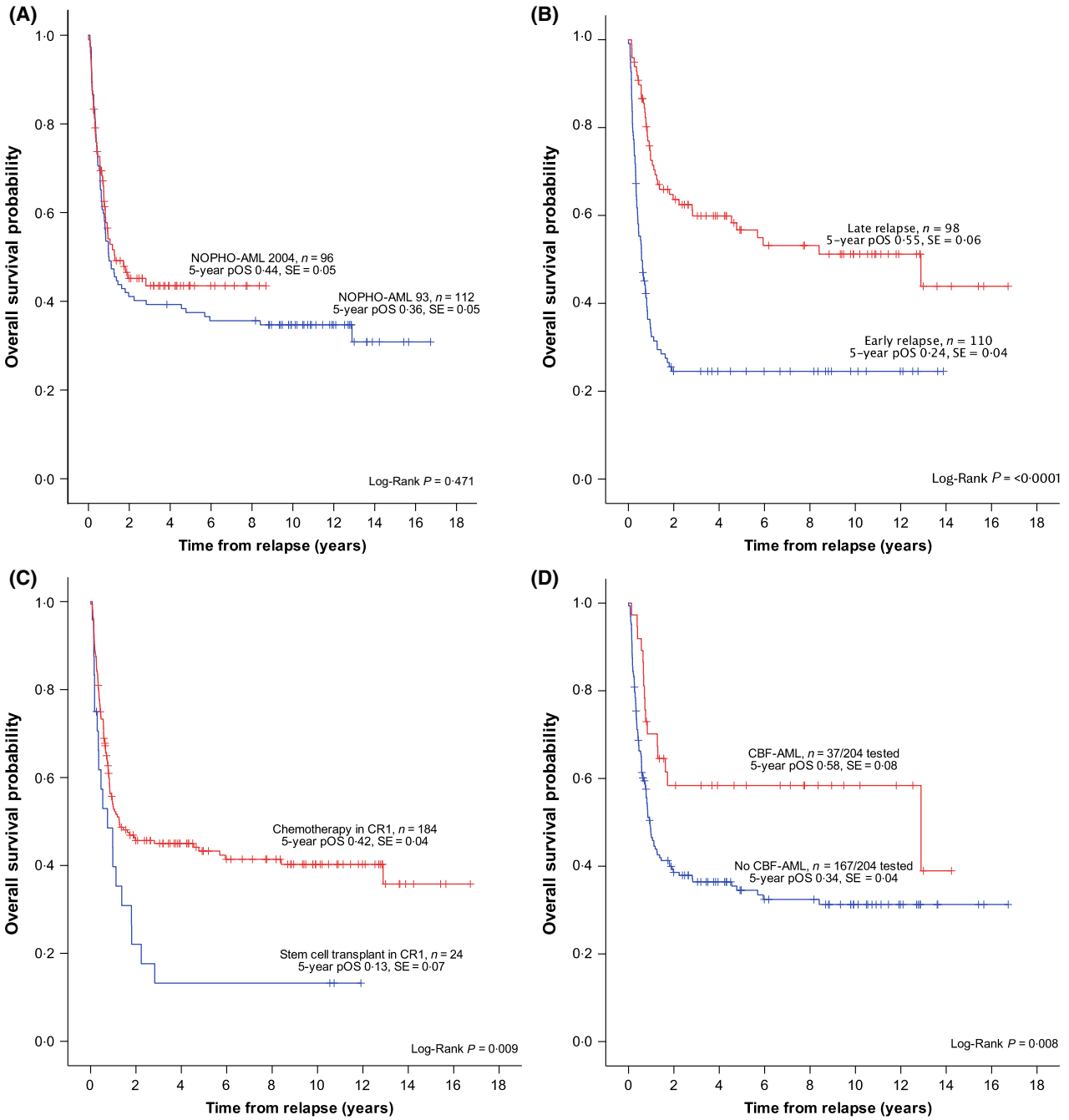

Fig 3. (A) Probability of survival according to initial therapy. (B) Probability of survival according to early relapse (<1 year from diagnosis) and late relapse (≥1 year from diagnosis). (C) Probability of survival according to consolidation therapy in first complete remission (CR1). (D) Probability of survival according to CBF-AML. AML, acute myeloid leukaemia; CBF-AML, core binding factor acute myeloid leukaemia; CR1, first complete remission; NOPHO, Nordic Society for Paediatric Haematology and Oncology; pOS; probability of overall survival; SE, standard error.

in CR2 rate between the two protocols. A number of studies have shown that different re-induction regimens are effective in relapsed AML but so far no specific treatment has been proven to be superior (Abrahamsson *et al*, 2007; Sander *et al*, 2010). Therefore, it is important to include careful consideration of the cumulative effect of toxicity when choosing re-induction therapy. In this respect, FLAG-based courses have been shown to have a favourable toxicity profile in children with relapsed AML (Kaspers *et al*, 2013).

Only two children in the whole cohort who did not enter CR2 survived, and both had SCT as relapse therapy. Very few data regarding children who survive without achieving CR2 after AML relapse exist, but several reports show that SCT can be effective in some patients, even when minimal

Table III. Response rates for patients receiving different induction regimes for relapsed acute myeloid leukaemia.

Remission achieved	NOPHO Upfront	FLAG/FLAG+ FLA/FLA+	Other	Data missing	Total
Yes	23 (70%)	110 (76%)	7 (70%)	2 (100%)	142 (75%)
No	10 (30%)	34 (24%)	3 (30%)		47 (25%)
Total	33	144	10	2	189

NOPHO upfront was either ATEDox (cytarabine. etoposide, thioguanine, doxorubicin) or AIET (cytarabine, idarubicin, etoposide, thioguanine). FLA, fludarabine, cytarabine; FLAG, FLA with granulocyte colony-stimulating factor; FLA+, FLAG+, FLA and FLAG respectively with addition of idarubicin or liposomal daunorubicin.

Table IV. Cox regression analysis of factors influencing overall survival in patients with relapsed AML (n = 204).

Variable	Hazard ratio (95% CI)	*P*-value
Age: (years)		
<2 years	0·70 (0·46–1·05)	0·085
2–9 years	1·0	
≥10 years	1·08 (0·65–1·78)	0·773
Gender		
Male	0·95 (0·65–1·38)	
Female	1·0	0·770
Protocol		
NOPHO-AML 2004	1·02 (0·69–1·51)	
NOPHO-AML 93	1·0	0·917
Time to relapse		
<1 year (early)	3·35 (2·25–4·97)	<0·0001
≥1 year (late)	1·0	
Consolidation in CR1		
SCT	2·46 (1·43–4·23)	0·001
Chemotherapy	1·0	
CBF-AML		
Yes	0·54 (0·31–0·94)	0·030
No	1·0	

95% CI, 95% confidence interval; AML, acute myeloid leukaemia; CBF-AML, core binding factor acute myeloid leukaemia; CR1, first complete remission; NOPHO, Nordic Society for Paediatric Haematology and Oncology.

residual disease burden at time of transplant is high (Leung *et al*, 2012).

Duration of first remission continues to be one of the main prognostic factors for survival. In our study, patients with early relapse (<1 year from diagnosis) had a survival of only 26% compared to 56% in patients with late relapse (≥1 year from diagnosis) and time to relapse was the strongest independent prognostic factor. These results are consistent with all other recent reports (Aladjidi *et al*, 2003; Abrahamsson *et al*, 2007; Castellino *et al*, 2008; Sander *et al*, 2010).

As shown in other studies, patients who were treated with SCT in primary therapy had a considerably worse prognosis following relapse (Sander *et al*, 2010). This probably reflects that patients who relapse following SCT have more resistant disease although some patients with relapse early after SCT may only tolerate less intensive therapy. Furthermore, particularly during the NOPHO-AML 93 protocol, a large proportion of patients relapsing after SCT never received re-induction therapy because they were considered incurable at that time.

We attempted to analyse whether cytogenetic and FAB subgroups correlated with outcome. However, caution must be exerted when interpreting these data due to the small patient numbers. The genetic aberrations were determined at initial diagnosis. Although clonal evolution may occur, it has been shown that the main cytogenetic subgroups are retained at relapse (Bachas *et al*, 2014). The only cytogenetic subgroups with an impact on outcome in our study were CBF mutations, *RUNX1/RUNX1T1* and *CBF/MYH11*, which were associated with better outcome. A recently published review also demonstrated that CBF-AML had a favourable outcome (Zwaan *et al*, 2015). In our study, FAB-type M5 was a predictor of poor outcome in univariate analyses. St Jude Children′s research group (Rubnitz *et al*, 2007) demonstrated that M5 correlated with high risk of relapse in multivariate analyses. In contrast, a large BFM study (Sander *et al*, 2010) and earlier NOPHO studies failed to show that FAB-type M5 had an impact on survival (Abrahamsson *et al*, 2007). *KMT2A* rearrangement, which is associated with AML FAB-type M5, did not correlate with poor prognosis in our cohort.

Although comparative studies are lacking, it is generally accepted that SCT should be included in consolidation therapy of AML relapse if patients can tolerate the procedure. In support of this, the survival of the children receiving SCT in our study was 61%. There has been a debate whether selected patients with relapsed AML can be cured without SCT and several studies show long-term survival in some patients following consolidation therapy with chemotherapy only (Goemans *et al*, 2008; Sander *et al*, 2010). However, many of these studies were performed at a time when primary therapy was less intensive and relapse rates higher, suggesting that some relapses may have been less resistant to therapy. Indeed four of our 20 patients, all with late relapse, survived after receiving chemotherapy only. Three of these patients had SCT in first remission and one patient had AML with t(8;21) and a late extramedullary relapse.

Obviously, the difference in survival between patients receiving SCT or not in CR2 is very large but no true comparison can be performed due to the very large selection bias. For example, to enable transplantation, a donor has to be identified, the disease must be controlled until the time of transplant and the patient needs to be in general good condition. Other studies have reported similar results but also suffered from the same drawbacks with low patient numbers and selection bias (Abrahamsson *et al*, 2007; Sander *et al*, 2010). Given the present results with a survival around 60% in patients receiving SCT it is questionable if it is ethically justified to perform a randomized trial comparing SCT with chemotherapy. However, identification of novel targeted therapies may allow for future relapse therapy without SCT in selected patients. Also, early identification of molecular relapse combined with pre-emptive therapy could reduce overall treatment intensity and increase survival.

At present, we believe that intensive re-induction therapy followed by SCT in CR2 is the best available treatment for the majority of patients with relapsed paediatric AML. Our cohort demonstrated no difference in survival between children receiving MUD or MFD transplants, but again the numbers of patients are very small.

In summary, in an unselected population-based cohort, almost 40% of paediatric patients with relapsed AML can be cured with intensive re-induction therapy followed by SCT. There has been no obvious improvement in outcome in the later time period. Patients with late relapses treated without SCT in first remission have the best prognosis.

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Conflicts of Interest and Disclosures

The authors report no potential conflict of interest.

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