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## Relapsed childhood acute lymphoblastic leukemia in the Nordic countries: prognostic factors, treatment and outcome

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### ABSTRACT

Relapse is the main reason for treatment failure in childhood acute lymphoblastic leukemia. Despite improvements in the up-front therapy, survival after relapse is still relatively poor, especially for high-risk relapses. The aims of this study were to assess outcomes following acute lymphoblastic leukemia relapse after common initial Nordic Society of Paediatric Haematology and Oncology protocol treatment; to validate currently used risk stratifications, and identify additional prognostic factors for overall survival. Altogether, 516 of 2735 patients (18.9%) relapsed between 1992 and 2011 and were included in the study. There were no statistically significant differences in outcome between the up-front protocols or between the relapse protocols used, but an improvement over time was observed. The 5-year overall survival for patients relapsing in the period 2002-2011 was  $57.5 \pm 3.4\%$ , but  $44.7 \pm 3.2\%$  ( $P < 0.001$ ) if relapse occurred in the period 1992-2001. Factors independently predicting mortality after relapse included short duration of first remission, bone marrow involvement, age ten years or over, unfavorable cytogenetics, and Down syndrome. T-cell immunophenotype was not an independent prognostic factor unless in combination with hyperleukocytosis at diagnosis. The outcome for early combined pre-B relapses was unexpectedly poor (5-year overall survival  $38.0 \pm 10.6\%$ ), which supports the notion that these patients need further risk adjustment. Although survival outcomes have improved over time, the development of novel approaches is urgently needed to increase survival in relapsed childhood acute lymphoblastic leukemia.

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### Introduction

With advances in chemotherapy, hematopoietic stem cell transplantation (HSCT) and supportive care, long-term survival in childhood acute lymphoblastic leukemia (ALL) is now 85-90%.<sup>1,2</sup> Despite increasing concerns regarding treatment-related

mortality and second malignancies, the main reason for treatment failure is still relapse.<sup>3</sup> In the Nordic countries, the relapse rate was close to 40% between 1981 and 1993 and only 30% remained in long-term second remission.<sup>4</sup> Over the last two decades, the reported relapse rates have been 15-20%<sup>1-3,5,6</sup> in the developed countries and the overall survival after relapse approximately 40-70%, depending on the follow-up time and the risk groups involved.<sup>7-12</sup>

Since 1992, all children aged one year and over diagnosed with pre-B and T-cell ALL in the Nordic countries (Denmark, Finland, Iceland, Norway, Sweden) have been treated according to a common Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL protocol. Children with relapsed ALL, on the other hand, have been treated heterogeneously since there has not been a common NOPHO ALL relapse protocol. The most commonly used relapse protocols have been the high-risk (HR) arms of the NOPHO ALL-92 or ALL-2000 front-line protocols, the German Berlin Frankfurt Münster (BFM) ALL-REZ relapse protocols, and the Finnish Relapse in Acute Lymphoblastic Leukemia (RALLE) pilot protocol,<sup>15</sup> which was used mainly in Finland between 2004 and 2010. After 2009, the British Children's Cancer and Leukemia Group (CCLG) ALLR3 relapse protocol has also been used in the Nordic countries, but the International study for treatment of childhood Relapsed ALL (IntReALL) trial is expected to replace other relapse protocols in the near future.

Risk stratification at relapse is based on the time from initial diagnosis to relapse, the anatomic site of relapse, and immunophenotype.<sup>10-14</sup> In addition, the most recent relapse protocols have integrated therapy response with minimal residual disease (MRD) for further treatment adjustments.<sup>9,11,15,16</sup> But unlike risk stratification at primary diagnosis, cytogenetic aberrations, including MLL rearrangements<sup>17,18</sup> and hypodiploidy,<sup>19,20</sup> currently do not directly modify relapse treatment intensity. Patients meeting high-risk criteria at relapse are recommended to undergo allogeneic HSCT in CR2, but the indication for HSCT in patients with lower risk is still under dispute, although in most centers patients with high MRD after conventional re-induction are candidates for allogeneic HSCT.<sup>8,21</sup> Rigorous selection of patients for the most appropriate treatment intensity is important not only to minimize the

risk of subsequent relapses but also to minimize treatment-related toxicity and mortality.<sup>22-25</sup>

Relatively few studies of the long-term outcome after relapsed childhood ALL have been published. Our study cohort is population-based and includes over 500 patients treated according to common up-front protocols, making it, to our best knowledge, the largest of its kind. We hypothesize that thorough analysis of prognostic factors, validation of the current risk stratification and comparison of treatment modalities could be helpful in improving treatment for relapsed childhood ALL.

## Methods

### Study population and data collection

Information on all children aged 1.0-14.9 years at diagnosis (n=2735) treated according to the NOPHO ALL-92 (n=1644) and ALL-2000 (n=1091) protocols were extracted from the NOPHO ALL registry and patients that relapsed before January 1st 2012 were identified. In 516 (18.9%) patients, relapse occurred as a primary event, in 339 (20.6%) after ALL-92 treatment, and in 177 (16.2%) following ALL-2000 treatment. In total, 130 (4.8%) patients underwent allogeneic HSCT in CR1 of which 31 (23.8%) relapsed. Since patients that relapse after HSCT in CR1 differ substantially from patients treated with chemotherapy only, in baseline characteristics, treatment and outcome, they were excluded from all outcome analysis. Thus this report includes the 485 relapse patients who did not receive HSCT in CR1. The database was frozen on the 31st of December 2013 and this dataset was used for outcome analysis. In 95 patients for whom the registration of relapse treatment, therapy response, outcome or follow-up status was incomplete, data were acquired from the treating hospitals to supplement the registration. Data concerning genetic aberrations were centrally reviewed by the NOPHO cytogenetic group. Cytogenetic findings were divided into four groups. Unfavorable: hypodiploidy (modal chromosomal number <45), MLL rearrangements, t(9;22) BCR/ABL and t(1;19); Favorable: high hyperdiploidy (modal chromosomal number >50) and t(12;21); Other: iAMP21, dic(9;20), unspecified chromosomal abnormalities; and Normal/missing: 46XX/XY or missing values. See *Online Supplementary Appendix* for the definition of relapse and second complete remission. This study was approved by the Ethical Review Board in Stockholm and was conducted in accordance with the Declaration of Helsinki.

**Table 1.** Risk stratification by immunophenotype, the time from diagnosis to relapse and the anatomic site of relapse.

5y-OS ± s.e.% or alive/total	Pre-B			T-cell		
	iEM	combined	iBM	iEM	combined	iBM
Very early	<i>n=9</i> <i>6/9</i>	<i>n=4</i> <i>0/4</i>	<i>n=50</i> <i>22.0 ± 5.9%</i>	<i>n=18</i> <i>27.8 ± 10.6%</i>	<i>n=10</i> <i>30.0 ± 14.5%</i>	<i>n=12</i> <i>8.3 ± 8.0%</i>
Early	<b>n=44</b> <b>76.0 ± 6.6%</b>	<b>n=21</b> <b>38.0 ± 10.6%</b>	<i>n=67</i> <i>36.6 ± 6.0%</i>	<b>n=3</b> <b>1/3</b>	<i>n=3</i> <i>0/3</i>	<i>n=8</i> <i>4/8</i>
Late	<b>n=24</b> <b>82.0 ± 8.3%</b>	<b>n=43</b> <b>77.4 ± 6.7%</b>	<b>n=155</b> <b>60.3 ± 4.1%</b>	<b>n=2</b> <b>1/2</b>	<i>n=1</i> <i>1/1</i>	<i>n=3</i> <i>1/3</i>

Standard-risk group (in bold) and high-risk group (in italics) according to the IntReALL risk classification. The boxes include the total number of patients and the overall survival for each subgroup. For subgroups involving less than 10 patients, survival is presented as the proportion of patients alive within the subgroup at the end of the follow-up period instead of 5-year overall survival (OS) (± standard error) (s.e.). Isolated extramedullary relapses (iEM): relapses not involving the bone marrow, such as the central nervous system (CNS), testis, lymph nodes, mediastinum and skin. Combined relapses: co-existent bone marrow and extramedullary involvement. Isolated bone marrow relapses (iBM): bone marrow relapses without any extramedullary involvement. Very early relapses: occurring <18 months from primary diagnosis. Early relapses: occurring ≥18 months from diagnosis and <6 months after completion of primary therapy. Late relapses: occurring ≥6 months after completion of primary therapy. Eight patients with unknown immunophenotype were excluded from the survival analysis; very early iBM = 1, early iBM = 2, early iEM = 1, late iBM = 2, late iEM = 2.

**Treatment**

A detailed description of the risk groups and treatment used in the NOPHO ALL-92 and ALL-2000 protocols, as well as a comparison of the long-term results of these up-front ALL treatments have been published by Schmiegelow et al.<sup>1</sup> We categorized the relapse treatment into four groups: ALL-REZ BFM protocols (90, 95/96 and

2002), NOPHO ALL-92 and ALL-2000 HR arms used as relapse therapy, RALLE pilot and “other treatment”. The “other treatment” group included patients treated with combinations of protocols, the CCLG ALLR3 relapse protocol, Children’s Cancer Group (CCG) relapse protocols and non-protocol chemotherapy. None of the 5 patients with t(9;22) were treated with tyrosine kinase inhibitors.

**Table 2. Cox’s proportional hazards regression analysis of risk factors for overall survival after ALL relapse.**

Prognostic factors	N.	Unadjusted model HR (95% CI)	Adjusted model 1 HR (95% CI)	Adjusted model 2 HR (95% CI)
Very early relapse <sup>1</sup>	104	3.84 (2.82 – 5.24)***	3.67 (2.56 – 5.26)***	3.80 (2.64 – 5.48)***
Early relapse <sup>1</sup>	149	1.85 (1.37 – 2.51)***	2.33 (1.70 – 3.19)***	2.36 (1.73 – 3.24)***
Isolated bone marrow relapse <sup>2</sup>	300	1.91 (1.33 – 2.73)***	2.87 (1.97 - 4.19)***	2.98 (2.04 – 4.35)***
Combined relapse <sup>2</sup>	82	1.47 (0.93 – 2.31)	2.22 (1.39 - 3.52)**	2.31 (1.45 – 3.68)***
T-cell ALL <sup>3</sup>	60	2.25 (1.61 – 3.13)***	1.43 (0.97 - 2.11)	–
WBC ≥ 100 x 10 <sup>9</sup> /L at primary diagnosis <sup>4</sup>	72	1.70 (1.24 – 2.34)**	1.27 (0.69 - 1.79)	–
Male <sup>5</sup>	314	0.96 (0.74 - 1.24)	1.07 (0.82 - 1.41)	1.08 (0.82 – 1.42)
Age ≥ 10 years at primary diagnosis <sup>6</sup>	97	1.76 (1.32 - 2.34)***	1.73 (1.28 - 2.34)**	1.80 (1.33 – 2.44)***
Unfavorable cytogenetics <sup>7*</sup>	28	2.55 (1.61 – 4.06)***	1.86 (1.14 – 3.03)*	1.83 (1.12 – 2.98)*
Favorable cytogenetics <sup>7*</sup>	173	0.80 (0.58 – 1.09)	1.05 (0.74 – 1.48)	1.05 (0.75 – 1.49)
Other cytogenetics <sup>7*</sup>	123	1.18 (0.85 – 1.62)	1.08 (0.77 – 1.50)	1.15 (0.82 – 1.60)
Down syndrome <sup>8</sup>	17	2.07 (1.21 - 3.56)**	2.70 (1.51 – 4.82)**	2.63 (1.48 – 4.70)**
T-cell + WBC ≥100 x 10 <sup>9</sup> /L <sup>9</sup>	27	3.46 (2.23 – 5.38)***	–	2.38 (1.43 – 3.96)**
T-cell + WBC <100 x 10 <sup>9</sup> /L <sup>9</sup>	33	1.66 (1.05 – 2.64)*	–	1.01 (0.60 – 1.69)
Pre-B + WBC ≥100 x 10 <sup>9</sup> /L <sup>9</sup>	45	1.22 (0.79 – 1.86)	–	0.95 (0.61 – 1.49)
Initial risk group High Risk <sup>10</sup>	201	1.91 (1.38 - 2.64)***	–	–
Initial risk group Intermediate Risk <sup>10</sup>	154	1.10 (0.77 - 1.57)	–	–

Hazard ratio (HR) for death. Reference groups: <sup>1</sup>late relapse, <sup>2</sup>isolated extramedullary relapse, <sup>3</sup>pre-B ALL, <sup>4</sup>white blood cell count (WBC) <100 x 10<sup>9</sup>/L at primary diagnosis, <sup>5</sup>female, <sup>6</sup>age <10 years at primary diagnosis, <sup>7</sup>no detected or missing data on chromosomal abnormalities, <sup>8</sup>not Down syndrome, <sup>9</sup>pre-B + WBC <100 x 10<sup>9</sup>/L at primary diagnosis, <sup>10</sup>Standard Risk patients according to the NOPHO ALL-92 and ALL-2000 protocols. \*Unfavorable cytogenetics: MLL rearrangements n=7, hypodiploidy (modal chromosomal number <45) n=10, t(9;22) n=5, t(1;19) n=6; Favorable cytogenetics: high hyperdiploidy (modal chromosomal number >50) n=106, t(12;21) n=67. \*\*\*P<0.001, \*\*P<0.01, \*P<0.05.

**Table 3. Relapse treatment for patients initially treated according to the NOPHO ALL-92 and NOPHO ALL-2000 protocols.**

Relapse protocol	Total n. (%)	NOPHO ALL-92 (%)*	NOPHO ALL-2000 (%)*	CR2 rate (%)	HSCT in CR2** (%)	5-year OS ± s.e %	10-year OS ± s.e %
NOPHO HR arms	91	83 (26)	8 (5)	89 (98)	43 (47)	0.52 ± 0.05	0.45 ± 0.05
Standard-risk	57 (63)	53	4	57 (100)	27 (47)	0.70 ± 0.06	0.59 ± 0.07
High-risk	34 (37)	30	4	32 (94)	16 (47)	0.21 ± 0.07	0.21 ± 0.07
ALL-REZ BFM	289	198 (61)	91 (56)	261 (90)	113 (39)	0.57 ± 0.03	0.52 ± 0.03
Standard-risk	187 (65)	128	59	180 (96)	59 (32)	0.67 ± 0.04	0.63 ± 0.04
High-risk	102 (35)	70	32	81 (79)	54 (53)	0.37 ± 0.05	0.33 ± 0.05
RALLE***	41	4 (1)	37 (23)	38 (93)	23 (56)	0.38 ± 0.08	0.38 ± 0.08
Standard-risk	18 (44)	3	15	18 (100)	7 (39)	0.49 ± 0.12	0.49 ± 0.12
High-risk	23 (56)	1	22	20 (87)	16 (70)	0.30 ± 0.10	0.30 ± 0.10
Other treatment	64	39 (12)	25 (16)	53 (83)	28 (44)	0.37 ± 0.06	0.37 ± 0.06
Standard-risk	31 (48)	17	14	28 (90)	15 (45)	0.55 ± 0.10	0.55 ± 0.10
High-risk	33 (52)	22	11	25 (81)	13 (41)	0.21 ± 0.07	0.21 ± 0.07
Total number	485	324	161	441 (91)	207 (43)	0.52 ± 0.02	0.47 ± 0.02

NOPHO High Risk (HR) arms, ALL-REZ Berlin Frankfurt Münster (BFM) relapse protocols, Relapse in Acute Lymphoblastic Leukemia (RALLE) pilot protocol, “Other treatment”; combinations of protocols, the Children’s Cancer and Leukemia Group (CCLG) ALLR3 relapse protocol, Children’s Cancer Group (CCG) relapse protocols and non-protocol treatment. Second complete remission (CR2), hematopoietic stem cell transplantation (HSCT). OS: overall survival; s.e.: standard error. \*Proportion of relapsed patients within the primary protocol. \*\* Proportion of patients undergoing HSCT in CR2 within each relapse protocol. \*\*\* Patients with isolated extramedullary relapses were not enrolled in the study.

### Statistical analysis

Base-line variables were compared between the two up-front protocols using Fisher's exact tests for categorical variables and the Mann-Whitney U test for continuous variables. In all survival analyses, the time-scale was defined by the time of diagnosis of relapse. Ten patients were lost to follow up, all in CR2 at the time of last contact and with a median time of follow up of 8.2 years (range 1.1-12.2 years). The Kaplan-Meier method was applied for generating survival curves for event-free survival (EFS) and overall survival (OS). Log rank test was used for comparing survival across groups. Cox's proportional hazards regression models were used for analysis of prognostic factors, estimating hazard ratios (HR) with 95% confidence intervals. OS was defined as the period from relapse diagnosis to death from any cause and censoring occurred at the date of last known follow up. In the analysis of EFS, patients were censored at the time of occurrence of the following second events: last known follow up for patients alive in CR2, second relapse, second malignancy (SMN), death caused by resistant disease or re-induction failure or death of undefined cause for patients in CR2. Cumulative incidence curves of second events were estimated by accounting for the competing nature of the second events.<sup>26</sup> The methods used for analyzing HSCT patients are described in the *Online Supplementary Appendix*. All tests were two-sided.  $P < 0.05$  were considered statistically significant. SPSS Statistics software version 21.0 and STATA version 13 were used for all statistical analyses.

## Results

### Patients' characteristics and second events

The characteristics and second events among patients with ALL relapse are listed in *Online Supplementary Tables S1 and S2*. Of the 103 patients with isolated extramedullary relapses, 72 had isolated CNS relapses, 17 isolated testicular, three combined CNS and testicular, and 11 included other extramedullary sites (*Online Supplementary Tables S2*). In total, 134 patients (28%) had CNS involvement at relapse of which 30 (22%) were T-cell lineage. Of the 104 patients with very early relapses, 89 (86%) were initially stratified as high or greater [68% if classified as high-risk according to the National Cancer

Institute (NCI) risk groups]. The only statistically significant differences in the relapse pattern between the up-front protocols were the distribution of cytogenetic findings (which can largely be explained by improvements in the detection methods), and the distribution of second events (which can partly be explained by the shorter follow-up time for the ALL-2000 patients). Subsequent relapse was the most common second event, occurring in 38% of patients. Second malignancies occurred in 11 patients of which 7 had undergone HSCT in CR2.

To validate the commonly used risk classification systems, we retrospectively assigned relapse risk groups according to the criteria in the new IntReALL trial and compared the overall survival between the groups (Table 1). This risk-grouping is based on the CCLG and the BFM risk categories. Patients with early combined pre-B ALL relapses were stratified as standard-risk but the 5 year-OS was only  $38.0 \pm 10.6\%$  (standard error, s.e.). Fifteen of these 21 patients underwent HSCT in CR2.

In total, 57 patient with Down syndrome were treated according to the NOPHO ALL-92 and ALL-2000 protocols of which 18 relapsed (32%): 17 after chemotherapy only and one after HSCT in CR1. Twelve were initially treated with the NOPHO ALL-92 protocol and 6 with the ALL-2000 protocol. Fifteen patients were categorized as standard-risk at relapse, but even though 14 of them reached CR2 and 3 subsequently underwent HSCT in CR2, only 3 survived long term. All deaths occurred after second relapse.

### Prognostic factors

Since we were studying a large cohort, we were able to include a number of variables in the regression analysis. The results of the Cox's proportional hazards regression analysis for overall survival are presented in Table 2. Primary risk groups were not included in the adjusted models since we adjusted for base-line characteristics at diagnosis. In the univariate analyses, age ten years or over at primary diagnosis, T-cell immunophenotype, short time in CR1, hyperleukocytosis at primary diagnosis, isolated bone marrow relapse, and unfavorable cytogenetics were adverse prognostic factors. In the first adjusted model,

**Table 4.** Cox's proportional hazards regression analysis of risk factors for overall survival in patients stratified as standard-risk at acute lymphoblastic leukemia relapse with HSCT in CR2 as a time-dependent covariate.

Prognostic factors	HSCT in CR2 /total (%)	Unadjusted model HR (95% CI)	Adjusted model HR (95% CI)
HSCT in CR2 <sup>1</sup>	108/283 (38%)	2.94 (1.90 – 4.53)***	2.82 (1.80 – 4.53)***
Male <sup>2</sup>	68/179 (38%)	0.70 (0.47 – 1.04)	1.29 (0.85 – 1.94)
Age $\geq$ 10 years at primary diagnosis <sup>3</sup>	28/46 (61%)	1.76 (1.10 – 2.81)*	1.39 (0.85 – 2.29)
WBC $\geq$ $100 \times 10^9/L$ at primary diagnosis <sup>4</sup>	11/21 (52%)	1.11 (0.54 – 2.30)	1.02 (0.49 – 2.15)
Unfavorable cytogenetics <sup>5</sup>	2/8 (25%)	2.17 (0.91 – 5.20)	2.15 (0.88 – 5.25)
Favorable cytogenetics <sup>5</sup>	33/117 (28%)	0.89 (0.56 – 1.43)	1.11 (0.68 – 1.83)
Other cytogenetics <sup>5</sup>	27/63 (43%)	1.23 (0.73 – 2.10)	1.27 (0.74 – 2.16)

Hazard ratio (HR) for death. Number of patients included in regression models  $n=283$ , number of observations  $n=417$ . Patients that did not achieve CR2 were excluded from the model ( $n=10$ ). Reference groups: <sup>1</sup>chemotherapy only; <sup>2</sup>female; <sup>3</sup>age  $<10$  years at primary diagnosis; <sup>4</sup>white blood cell count (WBC)  $<100 \times 10^9/L$  at primary diagnosis; <sup>5</sup>missing data or no detected chromosomal abnormalities. Unfavorable cytogenetics: MLL rearrangements, hypodiploidy (modal chromosomal number  $<45$ ),  $t(9;22)$ ,  $t(1;19)$ . Favorable cytogenetics: high hyperdiploidy (modal chromosomal number  $>50$ ) and  $t(12;21)$ .



**Table 5.** Reported outcomes of trials and cohorts in relapsed childhood acute lymphoblastic leukemia.

Relapse treatment	Relapse period	Risk group or type of relapse	N. of patients	Survival ± s.e.
<b>Non-uniform relapse treatment</b>				
<sup>a</sup> NOPHO <sup>4</sup>	1981-1993	All risk groups	315	11-year OS 33% ± 3 11-year EFS 28% ± 3
NOPHO	1992-2001	All risk groups	246	5-year OS 45% ± 3 5-year EFS 36% ± 3
NOPHO	2002-2011	All risk groups	239	5-year OS 58% ± 3 5-year EFS 51% ± 3
<sup>b</sup> COG <sup>29</sup>	1988-2002	All risk groups	1961	5-year OS 36% ± 2
<sup>c</sup> COG <sup>28</sup>	1996-2003	All risk groups	347	3-year OS 56% ± 3 3-year EFS 45% ± 3
<b>Non-randomized trials</b>				
MRC UKALL R1 <sup>49</sup>	1991-1995	All risk groups	256	5-year EFS 46% ± 3
CCLG ALL R2 <sup>7</sup>	1995-2002	All risk groups	150	5-year OS 56% ± 4 5-year EFS 47% ± 4
RALLE Pilot <sup>13</sup>	2004-2010	BM involving	40	5-year OS 37% ± 8 5-year EFS 37% ± 8
<sup>d</sup> COPRALL-97 <sup>50</sup>	1997-2002	Pre-B iEM relapses	68	
Time in CR1 <24 months			35	4-year OS 40% ± 8 4-year EFS 31% ± 8
Time in CR1 ≥24 months			34	4-year OS 76% ± 7 4-year EFS 61% ± 8
ALL-REZ BFM 95/96 <sup>9</sup>	1995-2001	<sup>e</sup> Intermediate risk		
MRD <10 <sup>-3</sup> after induction			46	10-year OS 91% ± 4 10-year EFS 76% ± 6
MRD ≥10 <sup>-3</sup> after induction			34	10-year OS 32% ± 8 10-year EFS 18% ± 7
ALL-REZ BFM 2002 <sup>8</sup>	2002-2009	<sup>e</sup> Intermediate risk	208	
Continuation chemotherapy if MRD <10 <sup>-3</sup> after induction			109	8-year OS 73% ± 7 8-year EFS 70% ± 5
HSCT in CR2 if MRD ≥10 <sup>-3</sup> after induction			99	8-year OS 68% ± 5 8-year EFS 64% ± 5
<b>Trials with randomizations</b>				
ALL-REZ BFM 87 <sup>10</sup>	1987-1990	All risk groups	183	15-year OS 37% ± 3 15-years EFS 30% ± 3
Timing of ARA-C and MTX during induction		Very early and early BM involving relapses	41	NS
ALL-REZ BFM 90 <sup>12</sup>	1990-1995	All risk groups	525	10-year OS 36% ± 2 10-year EFS 30% ± 2
MTX 1g/m <sup>2</sup> /36 hours vs. MTX 5g/m <sup>2</sup> /24 hours		Pre-B iEM, pre-B early and late relapses	269	NS
CCLG ALL R3 <sup>11</sup>	2003-2009	All risk groups	212	3-year OS 57% ± 4 3-year PFS 50% ± 4
Idarubicin in induction		All risk groups	109	3-year OS 45% ± 5 3-year PFS 36% ± 5
Mitoxantrone in induction		All risk groups	103	3-year OS 69% ± 5 3-year PFS 65% ± 5

Nordic Society of Paediatric Haematology and Oncology (NOPHO), Children's Oncology Group (COG), Medical Research Council (MRC), Children's Cancer and Leukemia Group (CCLG), Relapse in Acute Lymphoblastic Leukemia (RALLE) pilot, Berlin Frankfurt Münster (BFM). CR1: first complete remission; MRD: minimal residual disease; HSCT in CR2: hematopoietic stem cell transplantation in second complete remission; ARA-C: cytarabine; MTX: methotrexate; BM: bone marrow; iEM: isolated extramedullary. Very early relapse: occurring <18 months from primary diagnosis. Early relapse: occurring ≥18 months from diagnosis and <6 months after completion of primary therapy. Late relapse: occurring ≥6 months after completion of primary therapy. OS: overall survival; EFS: event-free survival; pFS: progression-free survival; NS: no statistically significant difference. If standard error (s.e.) was not available it was derived from the reported 95% confidence interval. <sup>a</sup>Includes patients who underwent HSCT in CR1 (n=65, 45 allogeneic and 19 autologous). In 1993, 6 NOPHO patients relapsed and are were included in both studies involving the 1981-1993 and 1992-2001 periods. <sup>b</sup>Patients enrolled in Children's Cancer Group (CCG) trials 1988-2002 for initial treatment but non-uniform relapse treatment. <sup>c</sup>Patients enrolled in the CCG-1952 study for initial treatment, only standard risk ALL (WBC <50x10<sup>9</sup>/L and age 1 to 9 years) but non-uniform relapse treatment. <sup>d</sup>Patients with early relapses (time in CR1 <24 months) were treated with VANDA induction and block therapy followed by HSCT in CR2 if donor available but patients with late relapses (time in CR1 ≥24 months) were treated with block therapy followed by radiotherapy and maintenance. <sup>e</sup>Intermediate risk (S2): pre-B early or late combined bone marrow relapse, pre-B late isolated bone marrow relapse, pre-B or T-cell very early and early isolated extramedullary relapse.

time to relapse (worse if earlier), site of relapse (worse if involving the bone marrow), age ten years or over at primary diagnosis, unfavorable cytogenetics and Down syndrome were all statistically significant independent prognostic factors. Adding up-front or relapse protocol to the adjusted model did not generate significant HRs or result in any notable change in the HRs of the other co-variables in the models. Immunophenotype is commonly used in the risk assessment at relapse, but although immunophenotype was a risk factor in the univariate analysis, it was not in the multivariate analysis. In the second adjusted model, we added an interaction variable combining immunophenotype and WBC at diagnosis and found that T-cell lineage disease with hyperleukocytosis at primary diagnosis ( $n=27$ ; 24 high-risk, 3 standard risk) was a notable independent risk factor for survival after relapse, HR 2.38 (95%CI: 1.43-3.96;  $P=0.001$ ). We analyzed separately the standard-risk group and adjusted for sex, age, Down syndrome and cytogenetics (*data not shown*). For the standard-risk group, age ten years or over HR 1.99 (1.24-3.21;  $P=0.004$ ) and Down syndrome, HR 4.70 (2.46-8.94;  $P<0.001$ ) were both independent prognostic factors for overall survival.

### Survival analysis

In the whole study population, the 5-year EFS was  $43.7\pm 2.3\%$  and the 5-year OS was  $51.5\pm 2.3\%$ . At five years, the EFS for the ALL-92 patients was  $42.1\pm 2.7\%$  and the OS was  $49.8\pm 2.8\%$  but  $46.8\pm 4.2$  and  $52.7\pm 4.4\%$  for the ALL-2000 patients, respectively. To investigate if there was a generally improved prognosis over time, the patients were divided into two relapse periods, 1992-2001 ( $n=239$ ) and 2002-2011 ( $n=246$ ), approximately corresponding to the timing of the introduction of more general MRD measurements in the Nordic countries.

Both OS and EFS were markedly higher for patients who relapsed between the years 2002-2011 compared to 1992-2001 (Figure 1A and B). HR for death was 0.62 (0.48-0.80;  $P<0.001$ ) for 2002-2011 but for second events the HR was 0.64 (0.51-0.82;  $P<0.001$ ). We compared the cumulative incidence of second events between the two relapse periods and found a reduction of second relapses in the later period (Figure 1C). There were no statistically significant differences between the time periods for the other second events. Looking for a possible explanation for these differences, we compared the pattern of relapse between the two time periods and observed a difference in the time distribution of relapses (*Online Supplementary S3*). In 1992-2001, 26.8% of relapses occurred very early, 32.6% early, and 40.6% late, but between 2002 and 2011, 16.3% occurred very early, 28.9% early, and 54.9% late ( $P=0.002$ ).

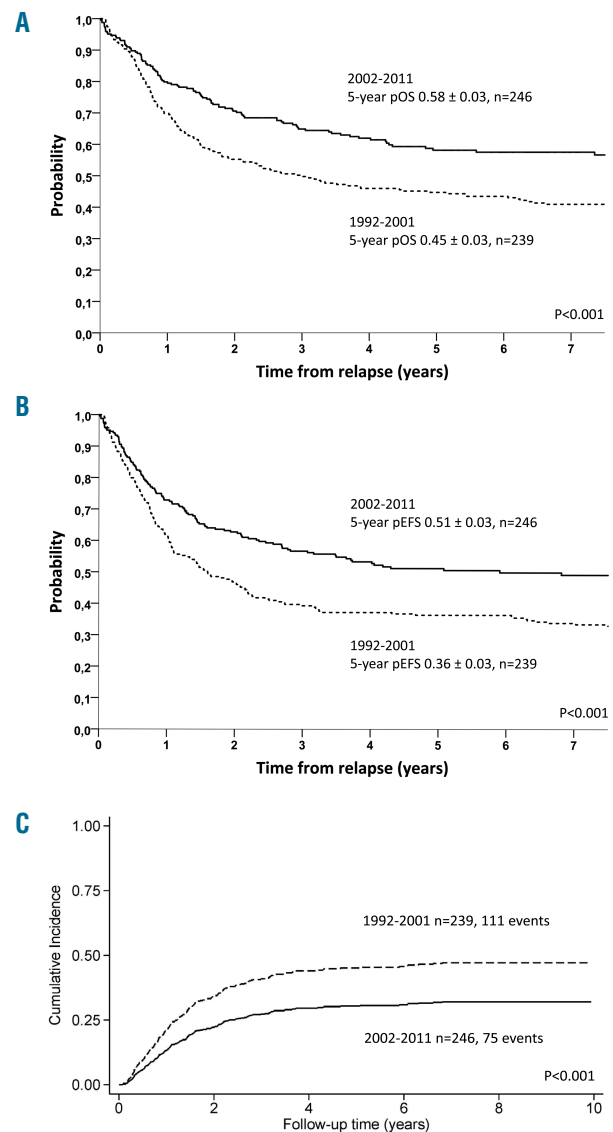
### Relapse treatment

The ALL-REZ BFM protocols were the most commonly used treatment for ALL relapse (60%) (Table 3). The proportion of patients receiving BFM treatments was relatively stable over the whole study period but the proportion of patients receiving NOPHO treatment was much lower in the later part of the study period (no patient after 2005). In addition, 8 patients were treated with the CCLG's ALLR3 protocol 2009-2011. The CR2 rate for the whole study period was 91%: 97% for standard-risk relapses and 82% for high-risk relapses. The CR2 rate for isolated extramedullary relapses was 95% and 90% for

bone marrow relapses, but only 71% for very early bone marrow relapses, compared to 97% for late bone marrow relapses. There was no significant difference in CR2 rate between the two primary protocols, relapse periods or specific relapse protocols. We did not observe a statistically significant difference in overall survival between the relapse protocols used during the study period (Table 3).

### Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation in CR2 was performed in 207 of the 485 patients (43%) included in the study: 137 of 324 ALL-92 patients (42%) and 70 of 161 ALL-2000 patients (44%). The allocation to HSCT was 43% (102 of 239) during 1992-2001 and 43% (105 of 246) during 2002-2011. The proportion of standard-risk patients allocated to HSCT was slightly higher in the period 1992-2001 (41% vs. 34%) but the proportion of high-risk patients allocated to HSCT increased from 45%



**Figure 1. Comparison of relapse periods 1992-2001 and 2002-2011.** (A) Overall survival after relapse. (B) Event-free survival after relapse. (C) Cumulative incidence of second relapse using competing risks.

during the period 1992-2001 to 61% during the period 2002-2011.

As expected, OS for high-risk patients was markedly higher if HSCT was performed in CR2,  $46.7 \pm 5.1\%$  compared to  $25.0 \pm 6.0\%$  ( $P < 0.001$ ). Interestingly, we observed the opposite association in the standard-risk group in which the overall survival was  $61.1 \pm 4.8\%$  for patients allocated to HSCT in CR2 compared to  $74.5 \pm 3.6\%$  ( $P = 0.02$ ) if continuation chemotherapy was used for consolidation. We investigated the effect of HSCT in CR2 on survival in the standard-risk group further in a time-dependent regression model and found a HR for the HSCT group of 2.94 (95%CI: 1.90-4.53) (Table 4). Adjusting for other non-stratifying base-line variables neither changed the HRs significantly (2.82; 95%CI: 1.80-4.41) nor yielded additional co-variables with a statistically significant and independent association with prognosis. Since there were only 3 patients with T-cell immunophenotype plus hyperleukocytosis in the standard-risk group, this variable was not included in the model as a single co-variate. Of the 27 patients with T-cell disease and hyperleukocytosis, 13 underwent HSCT in CR2 but only 4 survived (15%).

## Discussion

Relapse of ALL is still one of the most common childhood cancer subgroups with an incidence similar to rhabdomyosarcoma and neuroblastoma (Wilms tumor).<sup>27</sup> Despite the vast improvement in outcome after up-front ALL treatment, the increase in survival after relapse has not been as pronounced.<sup>4,7,10-12,28</sup> In this study, an improvement in OS over time was observed and is now close to 60%. Table 5 summarizes the reported results in relapsed childhood ALL from multicenter trials and cohorts over the last three decades.

At present, patients with relapsed ALL are allocated to different risk groups based on the immunophenotype, the time from primary diagnosis to relapse and the anatomic site of relapse. But unlike the risk stratification at primary diagnosis, cytogenetics are not used to individualize the treatment intensity. We demonstrate that unfavorable cytogenetics, age ten years or over, T-cell immunophenotype with hyperleukocytosis and Down syndrome were all additional individual prognostic factors in relapsed ALL.

The time from diagnosis to relapse is the strongest known individual risk factor for overall survival.<sup>4,29</sup> Nearly 90% of patients with very early relapses were stratified as high-risk or greater at initial diagnosis indicating that clinical and genetic features present at diagnosis affect survival after relapse.<sup>30,31</sup> In this study, two-thirds of the T-cell relapses occurred within 18 months, but contrary to previous findings, immunophenotype was not an individual prognostic factor for OS since it was over-ruled by other co-variables in the adjusted regression analysis. Interestingly, the interaction between WBC at diagnosis and T-cell immunophenotype created a strong prognostic variable. Only 4 out of 27 (15%) patients with T-cell immunophenotype and hyperleukocytosis survived long-term despite the use of HSCT in CR2 in 13 (48%) of them. However, this risk factor may be of limited additional value since with the current risk stratification, the majority of these patients are categorized as high-risk at relapse.

Children with Down syndrome have both an increased risk of developing ALL<sup>32</sup> and an increased risk of treat-

ment-related toxicity during primary ALL treatment.<sup>23-25,33</sup> Historically, Down syndrome ALL (DS-ALL) has been associated with inferior outcome, both with regard to OS and EFS.<sup>34,35</sup> In this study, DS-ALL was associated with very poor outcome, irrespective of the time period (early vs. late period) and the fact that most of these patients were stratified as standard-risk. Second relapses were the most common reason for treatment failure, indicating that patients with relapsed DS-ALL might have been treated with less intensive post-induction regimens to minimize the risk of treatment toxicity but subsequently failed to remain in long-term second remission.<sup>36</sup> In a study by Meyr *et al.*, children with DS had worse outcome after relapse mainly because of increased toxicity rather than subsequent relapse, but if the relapse occurred after the year 2000 this difference was not maintained.<sup>35</sup>

Adverse clinical factors, such as the time to relapse, age<sup>37,38</sup> and WBC<sup>39</sup> and cytogenetic risk factors,<sup>17,18,20</sup> are most likely surrogate markers for underlying submicroscopic genetic abnormalities.<sup>40-42</sup> With increased understanding of the biology of ALL, genetic factors are expected to be included in the future risk stratification and serve as targets for novel therapies.<sup>43-45</sup>

Despite the adjustments made to the NOPHO ALL-2000 protocol, OS did not differ significantly from the ALL-92 protocol: 5-year OS  $89.1 \pm 1.1\%$  and  $87.6 \pm 0.8\%$ , respectively.<sup>1</sup> Although the relapse rate was lower after the ALL-2000 treatment, it is expected that some of the late relapses from the ALL-2000 era are yet to occur. Although the pattern of relapse and outcome after relapse was very similar between the two NOPHO protocols, we observed a significant improvement in outcome for relapses occurring between 2002 and 2011 compared to 1992-2001, as well as a lower proportion of relapses generally associated with worse outcome (very early and early relapses) in the later period. In addition, we did not find a statistically significant difference in the CR2 rate or survival between the relapse protocols used during the study period, which supports the view that factors other than the protocol used explain the survival improvement and the changes in the relapse pattern between the two time periods. Minimal residual disease was measured in 73% of patients during the NOPHO ALL-2000 trial. However, although it was not used for risk stratification, it was optional to proceed to HSCT if MRD was 10-3 % or over after three months of treatment.<sup>1</sup> The retrospective study design and the lack of detailed MRD-data in our cohort constitute a drawback, but to estimate the effect of MRD on survival in general we compared outcomes before and after the year 2002, roughly coinciding with the general introduction of MRD analysis in most Nordic childhood cancer centers. The use of MRD in the assessment of treatment response after re-induction and preceding allogeneic HSCT in CR2 was obligatory in the ALL-REZ BFM 2002 and RALLE protocols. However, although not obligatory in the NOPHO ALL-2000 HR protocol, it was still available in many centers, since evidence at that time supported the stratification by MRD over morphology.<sup>46-48</sup> Therefore, after 2002 non-high-risk patients with high MRD levels after re-induction were recommended to undergo allogeneic HSCT in CR2 and a larger proportion of the high-risk patients was likely to be disease-free preceding HSCT.<sup>16,21</sup> The introduction of MRD could, therefore, be one of the explanations for the observed overall reduction of first and second relapses over time.

Our results indicate that patients stratified as standard-risk at relapse have worse OS after HSCT in CR2 compared to chemotherapy only. Although we adjusted for base-line variables such as age, WBC at diagnosis and cytogenetics, this survival difference remained clearly significant. However, this may reflect the fact that those patients selected for HSCT had a higher risk based on the MRD response after re-induction. This would result in a selection of patients with a higher risk of death to the HSCT group and MRD negative patients to the chemotherapy group. Previous studies have shown superior outcome after HSCT in CR2 for MRD positive standard-risk patients,<sup>8,9</sup> but in this study we did not find other risk factors or subgroups that seem to benefit from HSCT in CR2. Furthermore, the overall outcome of the SCT-group improved in the second time-period, when MRD was presumably available speaking against this type of negative selection (*data not shown*).

From 2015, the new international trial, IntReALL, will be the treatment of choice for relapsed childhood ALL in the Nordic countries. Our results indicate that the risk classification used in IntReALL is a reasonable approach, but we question whether early combined pre-B relapses should be classified as standard-risk instead of high-risk, since the 5-year overall survival for this subgroup was only 38.0% ( $\pm 10.6\%$ ), despite the fact that 14 of the 21 patients underwent HSCT in CR2. In the ALL-REZ BFM 2002 protocol, intermediate-risk patients with high MRD levels after re-induction have been recommended to undergo allogeneic HSCT in CR2 if a donor is available.

With these adjustments, the outcome for both good and poor responders has been similar (approximately 70% long-term OS), but the outcome for patients with early combined pre-B relapses has remained poor.<sup>8</sup>

## Conclusion

Over recent decades, improvements in the NOPHO ALL treatment have caused a reduction in the relapse rate. However, although improved survival over time was observed in this study, OS, especially for the high-risk patients, is still relatively poor. Most patients achieve second complete remission regardless of treatment protocol. But despite current treatment modalities, one-third of patients suffer second relapse. Therefore, better consolidation methods are needed without increasing the burden of treatment toxicities. Tailored risk-adapted treatment is the cornerstone of modern relapse therapy, but there is an urgent need for the development of new drugs and targeted therapies. There have been few reports on randomized controlled trials in patients with relapsed childhood ALL, and with the numbers of relapse patients decreasing, an international collaboration is very important to serve as a platform for progress to be made in the treatment of relapsed childhood ALL.

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## References

- Schmiegelow K, Forestier E, Hellebostad M, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. *Leukemia*. 2010;24(2):345-354.
- Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. *J Clin Oncol*. 2012;30(14):1663-1669.
- Stary J, Zimmermann M, Campbell M, et al. Intensive Chemotherapy for Childhood Acute Lymphoblastic Leukemia: Results of the Randomized Intercontinental Trial ALL IC-BFM 2002. *J Clin Oncol*. 2014; 32(3):174-184.
- Schroeder H, Garwicz S, Kristinsson J, Siimes MA, Wesenberg F, Gustafsson G. Outcome after first relapse in children with acute lymphoblastic leukemia: a population-based study of 315 patients from the Nordic Society of Pediatric Hematology and Oncology (NOPHO). *Med Pediatr Oncol*. 1995;25(5):372-378.
- Moricke A, Zimmermann M, Reiter A, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. *Leukemia*. 2010;24(2):265-284.
- Escherich G, Horstmann MA, Zimmermann M, Janka-Schaub GE. Cooperative study group for childhood acute lymphoblastic leukaemia (COALL): long-term results of trials 82,85,89,92 and 97. *Leukemia*. 2009;24(2):298-308.
- Roy A, Cargill A, Love S, et al. Outcome after first relapse in childhood acute lymphoblastic leukaemia - lessons from the United Kingdom R2 trial. *Br J Haematol*. 2005;130(1):67-75.
- Eckert C, Henze G, Seeger K, et al. Use of allogeneic hematopoietic stem-cell transplantation based on minimal residual disease response improves outcomes for children with relapsed acute lymphoblastic leukemia in the intermediate-risk group. *J Clin Oncol*. 2013;31(21):2736-2742.
- Eckert C, von Stackelberg A, Seeger K, et al. Minimal residual disease after induction is the strongest predictor of prognosis in intermediate risk relapsed acute lymphoblastic leukaemia - long-term results of trial ALL-REZ BFM P95/96. *Eur J Cancer*. 2013;49(6):1346-1355.
- Einsiedel HG, von Stackelberg A, Hartmann R, et al. Long-term outcome in children with relapsed ALL by risk-stratified salvage therapy: results of trial acute lymphoblastic leukemia-relapse study of the Berlin-Frankfurt-Munster Group 87. *J Clin Oncol*. 2005;23(31):7942-7950.
- Parker C, Waters R, Leighton C, et al. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. *Lancet*. 2010;376(9757):2009-2017.
- Tallen G, Ratei R, Mann G, et al. Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. *J Clin Oncol*. 2010;28(14):2339-2347.
- Saarinen-Pihkala UM, Parto K, Riikonen P, et al. RALLE pilot: response-guided therapy for marrow relapse in acute lymphoblastic leukemia in children. *J Pediatr Hematol Oncol*. 2012;34(4):263-270.
- Raetz EA, Borowitz MJ, Devidas M, et al. Reinduction platform for children with first marrow relapse of acute lymphoblastic Leukemia: A Children's Oncology Group Study[corrected]. *J Clin Oncol*. 2008;26(24):3971-3978.
- Paganin M, Zecca M, Fabbri G, et al. Minimal residual disease is an important predictive factor of outcome in children with relapsed 'high-risk' acute lymphoblastic leukemia. *Leukemia*. 2008;22(12):2193-2200.
- Coustan-Smith E, Gajjar A, Hijiya N, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia after first relapse. *Leukemia*. 2004;18(3):499-504.
- Forestier E, Johansson B, Gustafsson G, et al. Prognostic impact of karyotypic findings in childhood acute lymphoblastic leukaemia: a Nordic series comparing two treatment periods. For the Nordic Society of Paediatric Haematology and Oncology (NOPHO) Leukaemia Cytogenetic Study Group. *Br J Haematol*. 2000;110(1):147-153.
- Pui CH, Chessells JM, Camitta B, et al. Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. *Leukemia*. 2003;17(4):700-706.



19. Raimondi SC, Zhou Y, Mathew S, et al. Reassessment of the prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. *Cancer*. 2003;98(12):2715-2722.
20. Nachman JB, Heerema NA, Sather H, et al. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. *Blood*. 2007;110(4):1112-1115.
21. Bader P, Kreyenberg H, Henze GH, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. *J Clin Oncol*. 2009;27(3):377-384.
22. Saarinen-Pihkala UM, Heilmann C, Winiarski J, et al. Pathways through relapses and deaths of children with acute lymphoblastic leukemia: role of allogeneic stem-cell transplantation in Nordic data. *J Clin Oncol*. 2006;24(36):5750-5762.
23. Christensen MS, Heyman M, Mottonen M, Zeller B, Jonmundsson G, Hasle H. Treatment-related death in childhood acute lymphoblastic leukaemia in the Nordic countries: 1992-2001. *Br J Haematol*. 2005;131(1):50-58.
24. O'Connor D, Bate J, Wade R, et al. Infection-related mortality in children with acute lymphoblastic leukemia: an analysis of infectious deaths on UKALL2003. *Blood*. 2014;124(7):1056-1061.
25. Lund B, Asberg A, Heyman M, et al. Risk factors for treatment related mortality in childhood acute lymphoblastic leukaemia. *Pediatr Blood Cancer*. 2011;56(4):551-559.
26. Gray RJ. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. *The Annals of Statistics*. 1988;16(3):1141-1154.
27. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. *CA: A Cancer Journal for Clinicians*. 2014;64(2):83-103.
28. Malempati S, Gaynon PS, Sather H, La MK, Stork LC. Outcome after relapse among children with standard-risk acute lymphoblastic leukemia: Children's Oncology Group study CCG-1952. *J Clin Oncol*. 2007;25(36):5800-5807.
29. Nguyen K, Devidas M, Cheng SC, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. *Leukemia*. 2008;22(12):2142-2150.
30. Eckert C, Flohr T, Koehler R, et al. Very early/early relapses of acute lymphoblastic leukemia show unexpected changes of clonal markers and high heterogeneity in response to initial and relapse treatment. *Leukemia*. 2011;25(8):1305-1313.
31. Choi S, Henderson MJ, Kwan E, et al. Relapse in children with acute lymphoblastic leukemia involving selection of a preexisting drug-resistant subclone. *Blood*. 2007;110(2):632-639.
32. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. *Lancet*. 2000;355(9199):165-169.
33. Shah N, Al-Ahmari A, Al-Yamani A, Dupuis L, Stephens D, Hitzler J. Outcome and toxicity of chemotherapy for acute lymphoblastic leukemia in children with Down syndrome. *Pediatr Blood Cancer*. 2009;52(1):14-19.
34. Buitenkamp TD, Izraeli S, Zimmermann M, et al. Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. *Blood*. 2014;123(1):70-77.
35. Meyr F, Escherich G, Mann G, et al. Outcomes of treatment for relapsed acute lymphoblastic leukaemia in children with Down syndrome. *Br J Haematol*. 2013;162(1):98-106.
36. Bohnstedt C, Levensen M, Rosthøj S, et al. Physicians compliance during maintenance therapy in children with Down syndrome and acute lymphoblastic leukemia. *Leukemia*. 2013;27(4):866-870.
37. Mörücke A, Zimmermann M, Reiter A, et al. Prognostic Impact of Age in Children and Adolescents with Acute Lymphoblastic Leukemia: Data from the Trials ALL-BFM 86, 90, and 95. *Klin Padiatr*. 2005;217(06):310-320.
38. Forestier E, Schmiegelow K, Nordic Society of Paediatric Haematology and Oncology NOPHO. The Incidence Peaks of the Childhood Acute Leukemias Reflect Specific Cytogenetic Aberrations. *J Pediatr Hematol Oncol*. 2006;28(8):486-495.
39. Vaitkeviciene G, Forestier E, Hellebostad M, et al. High white blood cell count at diagnosis of childhood acute lymphoblastic leukaemia: biological background and prognostic impact. Results from the NOPHO ALL-92 and ALL-2000 studies. *Eur J Haematol*. 2011;86(1):38-46.
40. Yang JJ, Bhojwani D, Yang W, et al. Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. *Blood*. 2008;112(10):4178-4183.
41. Staal FJ, de Ridder D, Szczepanski T, et al. Genome-wide expression analysis of paired diagnosis-relapse samples in ALL indicates involvement of pathways related to DNA replication, cell cycle and DNA repair, independent of immune phenotype. *Leukemia*. 2010;24(3):491-499.
42. Bhojwani D, Kang H, Moskowitz NE, et al. Biologic pathways associated with relapse in childhood acute lymphoblastic leukemia: a Children's Oncology Group study. *Blood*. 2006;108(2):711-717.
43. Hogan LE, Meyer JA, Yang J, et al. Integrated genomic analysis of relapsed childhood acute lymphoblastic leukemia reveals therapeutic strategies. *Blood*. 2011;118(19):5218-5226.
44. Kuiper RP, Waanders E, van der Velden VH, et al. IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. *Leukemia*. 2010;24(7):1258-1264.
45. Chen IM, Harvey RC, Mullighan CG, et al. Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: a Children's Oncology Group study. *Blood*. 2012;119(15):3512-3522.
46. Panzer-Grumayer ER, Schneider M, Panzer S, Fasching K, Gadner H. Rapid molecular response during early induction chemotherapy predicts a good outcome in childhood acute lymphoblastic leukemia. *Blood*. 2000;95(3):790-794.
47. Biondi A, Valsecchi MG, Seriu T, et al. Molecular detection of minimal residual disease is a strong predictive factor of relapse in childhood B-lineage acute lymphoblastic leukemia with medium risk features. A case control study of the International BFM study group. *Leukemia*. 2000;14(11):1939-1943.
48. Donadieu J, Hill C. Early response to chemotherapy as a prognostic factor in childhood acute lymphoblastic leukaemia: a methodological review. *Br J Haematol*. 2001;115(1):34-45.
49. Lawson SE, Harrison G, Richards S, et al. The UK experience in treating relapsed childhood acute lymphoblastic leukaemia: a report on the medical research council UKALLR1 study. *Br J Haematol*. 2000;108(3):531-543.
50. Domenech C, Mercier M, Plouvier E, et al. First isolated extramedullary relapse in children with B-cell precursor acute lymphoblastic leukaemia: results of the Coopral-97 study. *Eur J Cancer*. 2008;44(16):2461-2469.