ORIGINAL ARTICLE

Risk group assignment differs for children and adults 1–45 yr with acute lymphoblastic leukemia treated by the NOPHO ALL-2008 protocol

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Abstract

Background: The prognosis of acute lymphoblastic leukemia is poorer in adults than in children. Studies have indicated that young adults benefit from pediatric treatment, although no upper age limit has been defined. *Design and methods:* We analyzed 749 patients aged 1–45 yr treated by the NOPHO ALL-2008 protocol. Minimal residual disease (MRD) on days 29 and 79, immunophenotype, white blood cell count (WBC), and cytogenetics were used to stratify patients to standard-, intermediate-, or high-risk treatment with or without hematopoietic stem cell transplantation. *Results:* Adults aged 18–45 had significantly lower WBCs at diagnosis compared with children aged 1–9 and 10–17 yr, but significantly more adults were stratified to high-risk chemotherapy (8%, 14%, 17%; $P < 0.0001$) or high-risk chemotherapy with transplantation (4%, 13%, 19%; $P < 0.0001$). This age-dependent skewing of risk grouping reflected more T-ALL (11%, 27%, 33%, $P < 0.0001$), poorer MRD response day 29 (MRD $< 0.1\%$: 75%, 61%, 52%; $P < 0.0001$), and more MLL gene rearrangements (3%, 3%, 10%; $P = 0.005$) in older patients. *Conclusions:* Even if identical diagnostics, treatment, and risk stratification are implemented, more adults will be stratified to high-risk therapy, which should be considered when comparing pediatric and adult outcomes.

Key words acute lymphoblastic leukemia; adults; adolescents; children; minimal residual disease; pediatric protocol

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Accepted for publication 25 February 2013   doi:10.1111/ejh.12097

Long-term survival is achieved in more than 80–90% of children with acute lymphoblastic leukemia (ALL) who are treated by the best contemporary protocols (1), whereas only 30–40% of adults with this disease are still alive 5 yr after diagnosis (1–7). This disparity in outcome may reflect a variety of factors, including leukemia biology [e.g., immunophenotype and cytogenetics (8)], tumor burden at diagnosis, the antileukemic agents included in the pediatric and adult protocols (9) and their age-dependent pharmacology (10, 11), the physicians' compliance with the protocol, and/or poor adherence to oral medication by the patients as they grow older (12). However, it has been difficult to evaluate the individual impact of these parameters, because protocols recruiting patients with a wide age range are lacking, and many reports have been burdened by potential bias in the interpretations. Thus, it remains to be determined whether adults and children with ALL diagnosed and treated according to the same protocol differ with regard to (i) presence of high-risk features at diagnosis, (ii) response to remission induction therapy and thus risk group assignment, (iii) risk of relapse and fatal toxicities within the risk group to which they were assigned, and (iv) adherence to treatment and the pharmacokinetics of the anticancer agents. To explore these aspects, six Nordic and Baltic countries designed a common protocol for Philadelphia chromosome-negative B-cell precursor ALL (BCP-ALL) or T-lineage ALL (T-ALL), which included patients 1–45 yr of age, who were all treated and assigned to risk groups according to identical criteria and prospectively registered in a common database. Here, we report that the use of standardized diagnostic and risk group assignment criteria resulted in significant skewing of adult patients toward higher-risk groups.

Design and methods

From July 2008 to December 2011, 796 consecutive patients in Sweden, Norway, Denmark, Finland (children only), Iceland (children only), and Lithuania who were diagnosed with Philadelphia chromosome-negative BCP or T-lineage ALL were registered in the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL-2008 database. Seventeen of those were excluded from the current analyses for the following reasons: treatment was being given according to the NOPHO ALL-2000 protocol ($n = 3$); diagnosis and initiation of treatment were done outside the Nordic/Baltic region ($n = 8$); ALL was a second cancer ($n = 2$); plasmocytoid dendritic cell ALL ($n = 3$); Charcot–Marie–Tooth disease resulted in vincristine intolerance ($n = 1$). Patients with Down syndrome ($n = 18$) or bilineage acute leukemia ($n = 7$) could be treated according to NOPHO ALL-2008 after certain modifications of the protocol, but those individuals were excluded from the present analysis. Finally, five patients were excluded because they had received more than 1 wk of antileukemic pretreatment (e.g., glucocorticosteroids) before entering the NOPHO ALL-2008 treatment protocol. Thus, 749 patients remained for analysis.

The diagnosis of ALL was confirmed by bone marrow biopsy/aspirates showing histology/cytomorphology and chemistry compatible with ALL ($\geq 25\%$ leukemic blasts in the bone marrow), immunophenotyping, G-band karyotyping, DNA index by flow cytometry, and targeted genetic analysis by fluorescent *in situ* hybridization and/or reverse transcriptase PCR for translocations t(9;22)(q34;q11)[*BCR-ABL*], t(1;19)(q23;p13), t(12;21) [*ETV6-RUNX1*], *RUNX1* amplification (ic21amp), dic(9,20), and 11q23 [*MLL*] gene aberrations.

Complete remission (CR) was defined as <5% leukemic blasts in the bone marrow confirmed by flow cytometric quantitation of residual leukemia (MRD). The regional or national ethics committees approved the protocol, and informed consent was obtained according to the Declaration of Helsinki, with Eudract number 2008-003235-02.

NOPHO ALL-2008 remission induction therapy and risk group stratification

The NOPHO ALL-2008 therapy is outlined in Table 1. Based on the characteristics of leukemia present at diagnosis and the response to remission induction therapy on days 15, 29, and 79 (or after HR-ALL block B1, see below), the patients were assigned to three risk groups: standard risk (SR), intermediate risk (IR), and high risk (HR). As an option, patients with a white blood cell count (WBC) $\geq 100 \times 10^9$/L could receive a 1–7-d prednisolone prephase until the WBC in peripheral blood was $<100 \times 10^9$/L, after which full induction therapy was to be given. Patients with central nervous system (CNS) involvement at diagnosis (i.e., leukemic blasts present in cytospin preparations of cerebrospinal fluid) received triple intrathecal therapy (TIT; prednisolone, methotrexate, and cytarabine) four times during the first 2 wk of the induction therapy.

Stratification 1 on treatment day 1

At diagnosis, patients were dichotomized to induction therapy with either dexamethasone 10 mg/m^2/d on days 1–21 (if T-ALL and/or WBC $\geq 100 \times 10^9$/L; Table 1) or prednisolone 60 mg/m^2/d on days 1–28 (all other patients). Patients who received dexamethasone and had a bone marrow aspirate containing more than 25% leukemic blasts (confirmed by flow cytometry) on day 15 were assigned directly to the HR block A1 from day 15.

Stratification 2 on treatment day 29

The second stratification took place on treatment day 29 according to the karyotype of the leukemic clone and the

Table 1 NOPHO ALL-2008 treatment days 1–92

	BCP-ALL and WBC $< 100 \times 10^9$/L	T-ALL and/or WBC $\geq 100 \times 10^9$/L	
Induction Days 1–29	Prednisolone 60 mg/m^2/day p.o. days 1–29 Vincristine 2.0 mg/m^2 i.v. days 1, 8, 15, 22, 29[1] Doxorubicin 40 mg/m^2 i.v. days 1, 22 Methotrexate i.t. days 1, 8, 15, 29[2]	Dexamethasone 10 mg/m^2/day p.o. days 1–21 The same as for BCP-ALL <100 $\times$ 10^9/L	

	Standard Risk (SR)	Intermediate Risk (IR)	High Risk (HR)

Consolidation Days 30–92 + HR blocks A, B Days 30–92	High-dose methotrexate
	  5 gr/m^2 i.v. days 36, 57, 78
	Vincristine 2.0 mg/m^2 i.v. days 43, 64[1]
	Pegylated asparaginase 1000 IU/m^2
	  i.m. days 30, 43, 57, 71, 85
	Methotrexate i.t. days 37, 58, 79[2]
	6-Mercaptopurine 25 mg/m^2
	  p.o. days 30–85
		The same as for SR except for CNS3[3]	Block A1
			  Cyclophosphamide 440 mg/m^2 i.v. days 1–5
			  VP16 100 mg/m^2 i.v. days 1–5
			  Pegylated asparaginase 1000 IU/m^2 i.m. day 6[4]
			  TIT i.t. (only in the beginning of A2 and A3
			Block B1 [5]
			  Dexamethasone 20 mg/m^2/day p.o. days 1–5
			  6-Mercaptopurine 100 mg/m^2 p.o. days 1–5
			  Vincristine 2.0 mg/m^2 i.v. days 1, 6[1]
			  High dose Ara-C 2.0 g/m^2/dose i.v. x 2 daily, days 5, 6
			  High dose methotrexate 5 g/m^2 i.v. day 1
			  Pegylated asparaginase 1000 IU/m^2 i.m. day 6
			  TIT i.t. day 1

BCP, B-cell precursor; WBC, white blood cell count; SR, standard risk; IR, intermediate risk; HR, high risk; p.o., per oral; i.m., intramuscular; i.v., intravenous; i.t., intrathecal; CNS, central nervous system; TIT, triple intrathecal therapy; VP16, etoposide.

[1]Maximum dose 2.5 mg for patients aged <18 yr and 2.0 mg for those $\geq$ 18 yr.

[2]Age-adjusted dose: 1.0–1.9 yr, 8 mg; 2.0–2.9 yr, 10 mg; $\geq$3.0 yr, 12 mg.

[3]If CNS3 involvement found at diagnosis, TIT (methotrexate, cytarabine, and prednisolone succinate) was given. CNS3 was defined as presence of one of the following: (i) $\geq$5 cells/μL cerebrospinal fluid compatible with leukemic blasts on cytospin, (ii) cranial nerve palsy, (iii) intracranial leukemic mass on MRI, and (iv) retinal involvement confirmed by MRI or biopsy (not mandatory).

[4]Unless given on day 30 of the induction treatment.

[5]Block B1 could be started when the absolute neutrophilic count was $\geq$ 0.5 x 10^{-9}/L and the platelet count was $\geq$80 $\times$ 10^{-9}/L and no sooner than 3 wk from the start of the previous block. For patients not in remission (bone marrow leukemic blast count $\geq$5%), the block could be started irrespective of the blood counts as soon as the patient's general condition allowed it.

bone marrow MRD status on day 29. Patients with t(1;19) (q23;p13), dic(9;20)(p13;q11), or ic21amp were assigned to IR therapy (unless MRD indicated HR therapy), and patients with *MLL* (11q23) mutations and/or hypodiploidy (i.e., <45 chromosomes or DNA index by flow cytometry <0.85) were assigned to HR therapy irrespective of their MRD response.

The patients who had received prednisolone induction therapy were assigned to SR or IR therapy based on whether their MRD on day 29 was <0.1% or 0.1–4.9%, respectively. Patients who had received dexamethasone induction therapy were assigned to IR or HR therapy depending on whether their day 29 MRD was <0.1% or 0.1–4.9%, respectively. Any patient with $\geq$5% MRD on treatment day 29 (or $\geq$5% MRD after block A1, if allocated to HR therapy on day 15) was assigned to HR therapy and hematopoietic stem cell transplantation (hSCT, see below). Patients were excluded from the SR group if (i) CNS3 involvement at diagnosis (leukocytes $\geq$5 $\times$ 10^6/L cerebrospinal fluid and leukemic blasts on cytospin preparation) and/or (ii) day 29 MRD quantitation was lacking or was not done within 50 d of the start of chemotherapy.

Stratification 3 on treatment day 79 or after block B1 and hematopoietic stem cell transplantation

Patients were eligible for HR-ALL chemotherapy and hSCT if their MRD response was $\geq$ 0.1% on treatment day 79 (SR- and IR-ALL), or after block B1 (HR-ALL). An MRD value $\geq$ 0.1 had to be confirmed within 1 wk by a second bone marrow aspirate. In addition, the treating center could choose hSCT as an option in cases with t(4;11)(11q23) [*MLL-AF4*] (adults only) or hypodiploidy. hSCT could be performed when MRD was at <0.1% and a donor was available.

Monitoring of minimal residual disease

MRD was measured by RQ-PCR-based techniques using patient-specific clonal Ig/TCR gene rearrangements according to the BIOMED-2 guidelines (13, 14) and/or by flow cytometry using protocol-defined six-color MRD panels for identification and monitoring of leukemia-associated immunophenotypes as defined by the NOPHO ALL-2000 guidelines (15). According to the NOPHO ALL-2008 protocol,

BCP-ALL MRD was quantified by flow cytometry, whereas for T-ALL, MDR-PCR assessment was applied.

Statistical analysis

To compare the prevalence of risk factors between subgroups, we used a chi-square or a Fisher's exact test for categorical analyses and a Mann–Whitney test for continuous variables as applicable. All tests were two-sided with $P < 0.05$ considered statistically significant. Spearman's correlation coefficient (r_s) was used to determine possible associations. All statistical analyses were performed using SPSS software version 19.0.0 (Chicago, IL, USA).

Results

Demographic data

A total of 412 males and 337 females representing 624 BCP-ALL and 125 T-ALL cases were eligible for analysis (Table 2). The frequency of T-ALL was significantly lower in patients aged 1–9 yr than in those aged 10–17 and 18–45 yr (11%, 27%, and 34%; $P < 0.0001$), but did not differ between the two oldest age groups ($P = 0.4$). Compared with females, more males had T-cell ALL ($P = 0.002$), and males were older at diagnosis (median: 5.5 vs. 5.0 yr; $P = 0.02$). Age and WBC at diagnosis were significantly

and inversely correlated in both BCP-ALL ($r_s = -0.2$, $P < 0.0001$) and T-ALL ($r_s = -0.3$; $P < 0.0001$). Among the BCP-ALL patients, the three age groups did not differ with regard to the proportion with WBC $\geq 100 \times 10^9$/L (7%, 12%, 10%, $P = 0.2$), whereas the number of T-ALL patients with WBC $\geq 100 \times 10^9$/L decreased significantly with increasing age (62%, 40%, 24%; $P = 0.003$) (Table 2).

Cytogenetics

The occurrence of t(12;21) ($n = 153$) and hyperdiploidy ($n = 215$) decreased with increasing age ($P < 0.0001$). All cases of dic(9;20) ($n = 11$) were found in patients younger than 9 yr (Table 2). In contrast, the frequency of t(4;11)/ *MLL-AF4* rearrangements ($n = 26$) was significantly higher in patients aged 18–45 yr (3%, 3%, 10%; $P = 0.005$, Table 2). Also, ic21amp ($n = 17$) was more common in patients aged 10–17 yr compared with all other age groups (7% vs. <2%, $P = 0.001$). The distribution of hypoloidy ($n = 11$) and t(1;19) ($n = 23$) did not differ significantly between the three age groups, but the number of patients with these karyotypes was low.

Risk group distribution

Figure 1 illustrates the risk group distribution on days 1, 29, and 79. The higher frequency of older patients stratified to

Table 2 Characteristics of the acute lymphoblastic leukemia (ALL) patients

	Total	1–9.9	10–17.9	18–45	P value (Fisher's exact test)
Age in years					
Median age		3	14	28	
Number of patients	749	515 (69%)	148 (20%)	86 (11%)	Overall
Sex					
Male	412 (55%)	267 (52%)	91 (62%)	54 (63%)	0.04
Female	337 (45%)	248 (48%)	57 (38%)	32 (37%)	
Phenotype					
B-ALL	624 (83%)	459 (89%)	108 (73%)	57 (66%)	
T-ALL	125 (17%)	56 (11%)	40 (27%)	29 (34%)	<0.0001
WBC, no. patients (%)					
Median count $\times 10^9$/L (range)		11.6 (0.4–1161)	9.8 (0.5–757)	12.6 (0.7–482)	
B-ALL < 100	572 (76%)	426/459 (93%)	95/108 (88%)	51/57 (90%)	
B-ALL $\geq$ 100	52 (7%)	33/459 (7%)	13/108 (12%)	6/57 (10%)	0.2
T-ALL < 100	67 (9%)	21/56 (38%)	24/40 (60%)	22/29 (76%)	
T-ALL $\geq$ 100	58 (8%)	35/56 (62%)	16/40 (40%)	7/29 (24%)	0.003
Cytogenetics, No. positive/No. tested (%)					
t(12;21)	153/737 (21%)	145/511 (28%)	7/146 (5%)	1/80 (1%)	<0.0001
hyperdiploidy $\geq$/= 50	215/630 (34%)	180/446 (40%)	24/113 (21%)	11/71 (16%)	<0.0001
t(4;11)/MLL	26/745 (3%)	13/514 (3%)	5/148 (3%)	8/83 (10%)	0.005
dic(9;20)	11/719 (2%)	11/494 (2%)	0/144 (0%)	0/70 (0%)	0.09
Ic21amp	17/730 (2%)	6/506 (1%)	10/147 (7%)	1/77 (1%)	0.001
hypodiploidy $\leq$ 44	11/701 (2%)	7/492 (1%)	3/138 (2%)	1/71 (1%)	0.7
t(1;19)	23/743 (3%)	15/513 (3%)	5/148 (3%)	3/82 (4%)	0.9

induction therapy with dexamethasone reflects the distribution of T-ALL ($P < 0.0001$). On day 15 (results not shown), information on bone marrow status was insufficient for 30 patients (14 of them older than 17 yr). Among the remaining 719 patients, the bone marrow blast count was $\geq 25\%$ in a larger proportion of those with BCP-ALL and WBC $\geq 100 \times 10^9$/L or T-ALL than in those with BCP-ALL and WBC $< 100 \times 10^9$/L (13% vs. 6%; $P = 0.004$). WBC $\geq 100 \times 10^9$/L and $\geq 25\%$ blasts on day 15 were found in 6%, 9%, and 7% of BCP-ALL patients in the age groups 1–9, 10–17, and 18–45 yr, respectively, and thus, as also noted for T-ALL, those fractions did not increase with increasing age ($P = 0.2$).

The complete remission (CR) rates measured on treatment day 29 ($n = 736$; 13 lacked data on morphological BM examination) were of 96%, 89%, and 86% ($P = 0.001$) for patients in the age groups 1–9, 10–17, and 18–45 yr, respectively. Of the 736 patients, 22 were allocated directly to HR treatment on day 15 because of $\geq 25\%$ bone marrow blasts; accordingly, there was no day 29 bone marrow information on these individuals, and thus, they were not considered to be in CR on day 29 in this analysis.

Considering the MRD response level (<0.1%, 0.1–5%, or $\geq 5\%$) on treatment day 29 ($n = 719$; 22 assigned directly to HR on day 15, one left the Nordic/Baltic region, and

seven died of treatment-related complications), we found a significant difference ($P < 0.0001$) between the age groups: a good response to treatment (MRD < 0.1%) was achieved in only 51% of patients aged 18–45 yr ($n = 44$) but was attained in 62% and 74% of those aged 10–17 yr ($n = 91$) and 1–9 yr ($n = 382$), respectively. The inverse correlation between age and the measured MRD level was highly significant for the 657 patients for whom such data were available ($P < 0.0001$, $r_s = -0.2$; Fig. 2).

The risk group distribution on day 29 (Fig. 3A) illustrates the highly significant skewing of older patients toward higher-risk groups, including eligibility for hSCT ($P < 0.0001$). At the final stratification on day 79 or after block B1 (Fig. 3B), the skewed association between age and risk group was even more pronounced ($P < 0.0001$). Figure 3C shows the various stratifying factors leading to a final assignment to HR chemotherapy in 75 patients. The majority of those patients were allocated to HR because of a poor response determined by MRD assessment on day 29 (51%), or the presence of *MLL* gene rearrangements or hypodiploidy (37%). A less common reason was the presence of more than 25% blasts in the bone marrow on day 15 (11%) in patients receiving dexamethasone induction. The distribution of HR criteria did not differ between the three age groups ($P = 0.1$).
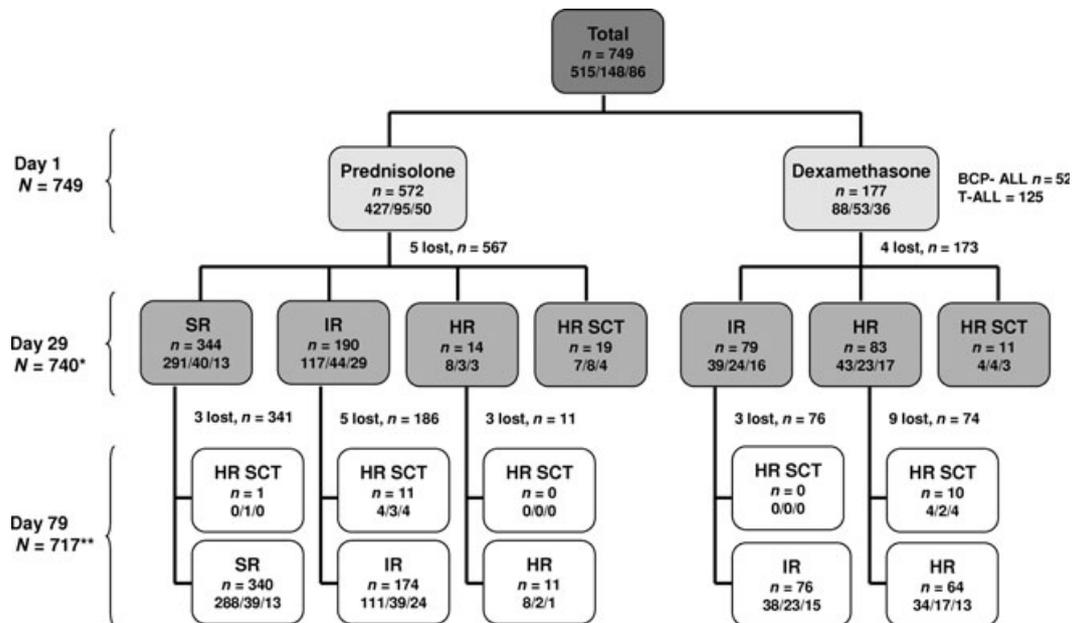

Figure 1 Flow diagram of risk group distribution and treatment assignment from day 1 to day 79. The number of patients in the different age groups is given according to age groups in the following order: 1.0–9.9 yr/10.0–17.9 yr/18–45 yr. *Day 29. During induction with prednisolone, five patients were lost to further analysis for the following reasons: one left the Nordic/Baltic region, one drug abuser neglected therapy, and three died of treatment-related complications. During induction with dexamethasone, four patients died from treatment-related complications. **Day 79. After induction with prednisolone, 11 patients were lost to further analysis: five had insufficient data registered, one left the Nordic/Baltic region, two received highly modified treatment due to infections, and three died of treatment-related complications. After induction with dexamethasone, 12 patients were lost to further analysis: one changed to another adult ALL protocol because of liver toxicity (clinician's decision), six had insufficient data, four died of treatment-related complications, and one had a bone marrow relapse.
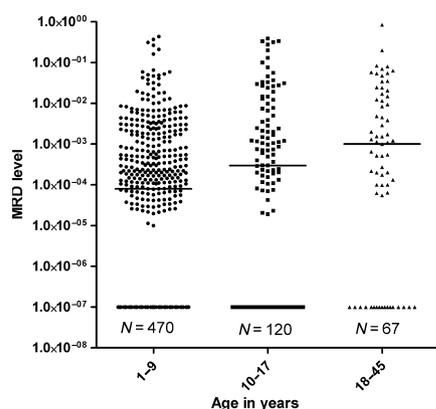
Figure 2 Exact levels of MRD on day 29 ($n = 657$; $P < 0.0001$). Horizontal lines depict median levels; patients with negative MRD are marked as 10^{-7}.

Hematopoietic stem cell transplantation

A total of 52 patients were eligible for hSCT based on MRD levels measured on day 29/post-A1 or day 79/post-B1. The primary indication was a high MRD level either on day 29 of treatment (65%, $n = 34$) or on day 79 or after block B1 treatment (29%, $n = 15$). Hypodiploidy ($n = 2$) and t (4;11) ($n = 1$) were the remaining indications. The distribution of hSCT criteria did not differ between the three age groups ($P = 0.9$; Fig. 3D).

Adherence to treatment

The scheduled duration of induction treatment was 29 d. The median interval from day 1 to day 29 was 28 d for all age groups (50% ranges in age groups 1–9, 10–17, and 18–45 yr: 28–29, 28–29, and 28–29 d, respectively, $P = 0.3$). No adult patients died during induction therapy. The median time from day 29 to day 79 for SR or IR patients was 54, 55, and 52 d in the age groups 1–9, 10–17, and 18–45 yr, respectively (50% ranges: 49–59, 49–61, and 49–57 d, $P = 0.3$).

Discussion

Studies that compare the survival of children and adults with ALL have assessed patients given different treatments, which makes it difficult to interpret the results. Such bias includes differences in the early response to chemotherapy and, thus, risk grouping. This bias was eliminated in the present NOPHO ALL-2008 protocol, because patients aged 1–45 yr had received the same induction treatment and were risk-grouped according to identical criteria. As adults have a higher frequency of T-ALL and HR cytogenetics and show less favorable response to induction treatment, the critical clinical question is really whether adults and children who are diagnosed and stratified according to identical criteria have different prognoses within each risk group. This question will

be addressed in the ongoing prospective evaluation of the Nordic/Baltic NOPHO ALL-2008 treatment protocol.

Several investigations have shown that MRD measurements are feasible in adult ALL patients and can serve as an important prognostic marker at certain time points (16–24). The poorer response to induction treatment seen in our data is reflected in both a lower CR rate and a reduced proportion of adult patients with MRD < 0.1% on day 29.

In the current cohort, the distribution of cytogenetic aberrations showed more t(12;21) and hyperdiploidy among children 1–9 yr of age, more Ic21amp among those aged 10–17 yr, and more MLL in patients older than 18 yr, which confirms observations made in other studies (25–28). Data on the frequency of Ic21amp in adults are still sparse, as this aspect has not been examined routinely in the past (29). Another noteworthy finding of our study, which can be only partly explained by the presence of T-ALL and *MLL* rearrangements, is that adults had a significantly poorer response to induction therapy compared with younger patients, even though the tumor burden at diagnosis was inversely correlated with age.

Questions have been posed concerning non-adherence to treatment by adult patients or their physicians (30–33). The identical actual duration of induction and consolidation therapy indicates that adults tolerate these treatment phases as well as children and that pediatricians and adult hematologists follow the protocol prescribed treatment intensity irrespective of their patients' age. In conclusion, we found that the CR rate, the MRD response, and risk group assignment differed significantly between pediatric and adult ALL patients treated according to a common protocol. These findings indicate that the biology of the disease changes with increasing age, and this may contribute to the poor treatment response seen in adult ALL patients. Further studies are needed to reveal whether a response-directed approach can improve the poorer long-term survival of adult patients.

Authorship

Contributions: NT designed the study, collected, analyzed, and interpreted data, wrote and edited the manuscript; KS designed the study, interpreted data, served as principal investigator, and critically reviewed the manuscript; HB designed the study, interpreted data, served as an investigator, and critically reviewed the manuscript; TWC performed statistical analyses and interpreted data; MH designed the study, served as investigator and data manager, collected data, and edited the manuscript; JA, PB, HH, LG, MSH, EH, HM, OGJ, OJN,
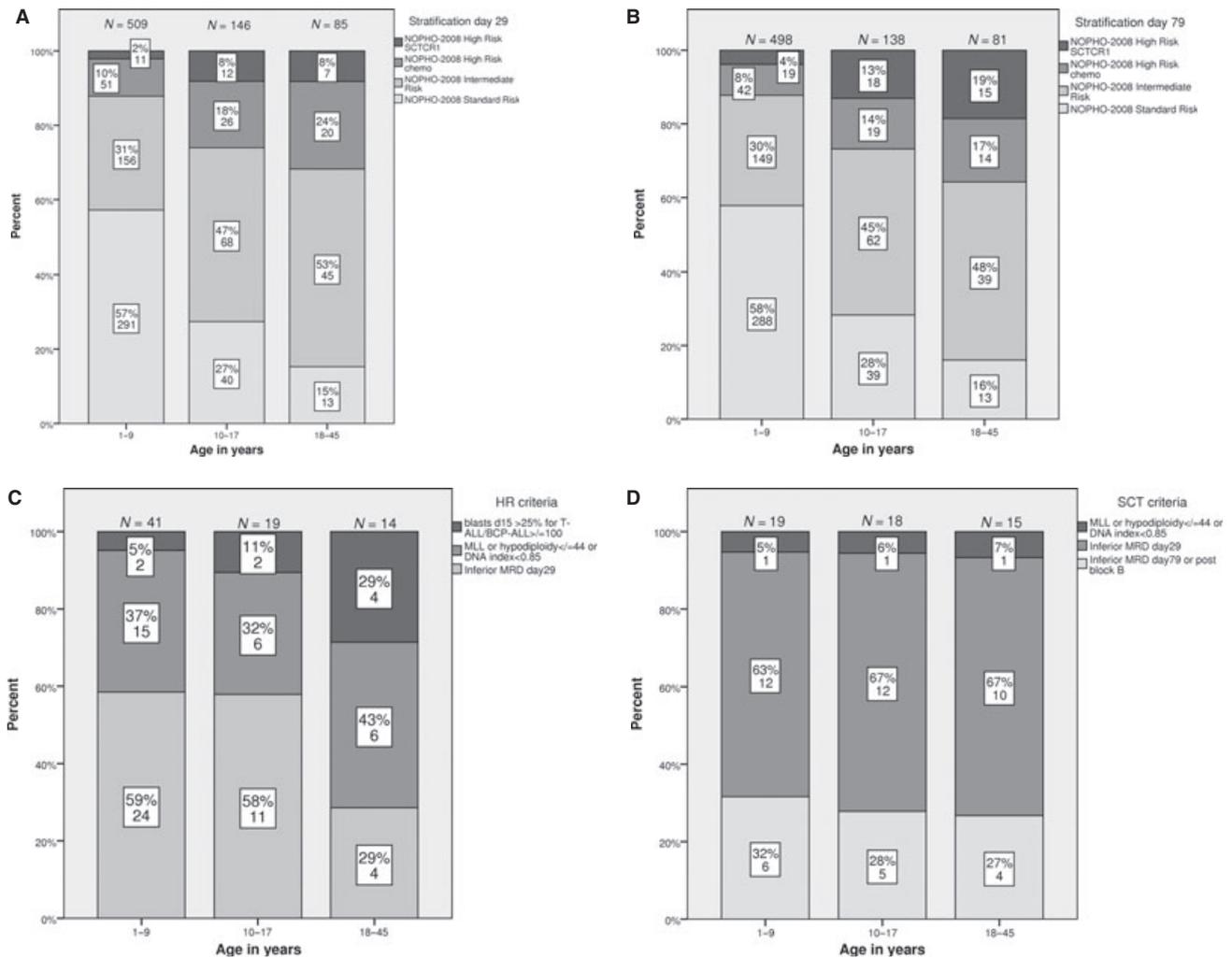
Figure 3 Risk group distribution on days 29 and 79, and criteria for treatment of high-risk with/without transplantation according to age group. (A) Risk group distribution on day 29 shown for 740 of 749 patients ($P < 0.0001$). The remaining nine were lost from analysis for the following reasons: one left the Nordic/Baltic region, one was non-compliant due to drug abuse, and seven died from treatment-related complications. (B) Risk group distribution on day 79 shown for a total of 717 patients ($P < 0.0001$). The remaining 32 patients could not be stratified for the following reasons: two left the Nordic/Baltic region, one was non-compliant due to drug abuse, 14 died of treatment-related complications, 11 had insufficient data registered, two received highly modified treatment due to infections, one changed to an adult protocol because of liver toxicity (clinician's decision), and one had a bone marrow relapse. (C) Criteria for treatment in the HR group shown in relation to age. The data represent 75 patients, including one-2-yr-old T-ALL patient who did not have MRD quantitation and was stratified to HR according to the protocol ($P = 0.1$). (D) Criteria for HR hSCT in first remission shown in relation to age ($n = 52$; $P = 0.9$).

PQP, MT, GV, KV, and AÅ were designed the study, served as investigator, collected data, and edited the manuscript. All authors approved the final manuscript.

Disclosure

The authors declare no competing financial interests.

References

1. Schmiegelow K, Forestier E, Hellebostad M, Heyman M, Kristinsson J, Soderhall S, Taskinen M. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. *Leukemia* 2010;**24**:345–54.

2. Chessells JM, Hall E, Prentice HG, Durrant J, Bailey CC, Richards SM. The impact of age on outcome in lymphoblastic leukaemia; MRC UKALL X and XA compared: a report from the MRC Paediatric and Adult Working Parties. *Leukemia* 1998;**12**:463–73.

3. Kantarjian HM, O'Brien S, Smith TL, *et al.* Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. *J Clin Oncol* 2000;**18**:547–61.

4. Labar B, Suciu S, Zittoun R, *et al.* Allogeneic stem cell transplantation in acute lymphoblastic leukemia and non-Hodgkin's

lymphoma for patients <or=50 years old in first complete remission: results of the EORTC ALL-3 trial. *Haematologica* 2004;**89**:809–17.

5. Rowe JM. Prognostic factors in adult acute lymphoblastic leukaemia. *Br J Haematol* 2010;**150**:389–405.

6. Thomas X, Boiron JM, Huguet F, *et al.* Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. *J Clin Oncol* 2004;**22**:4075–86.

7. Toft N, Schmiegelow K, Klausen TW, Birgens H. Adult acute lymphoblastic leukaemia in Denmark. A national population-based retrospective study on acute lymphoblastic leukaemia in Denmark 1998–2008. *Br J Haematol* 2012;**157**:97–104.

8. Secker-Walker LM, Prentice HG, Durrant J, Richards S, Hall E, Harrison G. Cytogenetics adds independent prognostic information in adults with acute lymphoblastic leukaemia on MRC trial UKALL XA. MRC Adult Leukaemia Working Party. *Br J Haematol* 1997;**96**:601–10.

9. Stock W, La M, Sanford B, Bloomfield CD, Vardiman JW, Gaynon P, Larson RA, Nachman J. What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. *Blood* 2008;**112**:1646–54.

10. Plasschaert SL, Vellenga E, de Bont ES, van der Kolk DM, Veerman AJ, Sluiter WJ, Daenen SM, de Vries EG, Kamps WA. High functional P-glycoprotein activity is more often present in T-cell acute lymphoblastic leukaemic cells in adults than in children. *Leuk Lymphoma* 2003;**44**:85–95.

11. Goker E, Lin JT, Trippett T, Elisseyeff Y, Tong WP, Niedzwiecki D, Tan C, Steinherz P, Schweitzer BI, Bertino JR. Decreased polyglutamylation of methotrexate in acute lymphoblastic leukemia blasts in adults compared to children with this disease. *Leukemia* 1993;**7**:1000–4.

12. Schmiegelow K, Heyman M, Gustafsson G, Lausen B, Wesenberg F, Kristinsson J, Vettenranta K, Schroeder H, Forestier E, Rosthoej S. The degree of myelosuppression during maintenance therapy of adolescents with B-lineage intermediate risk acute lymphoblastic leukemia predicts risk of relapse. *Leukemia* 2010;**24**:715–20.

13. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia* 2003;**17**:1013–34.

14. van Dongen JJ, Langerak AW, Bruggemann M, *et al.* Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia* 2003;**17**:2257–317.

15. Bjorklund E, Mazur J, Soderhall S, Porwit-MacDonald A. Flow cytometric follow-up of minimal residual disease in bone marrow gives prognostic information in children with acute lymphoblastic leukemia. *Leukemia* 2003;**17**:138–48.

16. Bassan R, Spinelli O, Oldani E, *et al.* Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). *Blood* 2009;**113**:4153–62.

17. Bruggemann M, Raff T, Flohr T, *et al.* Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood* 2006;**107**:1116–23.

18. Campana D. Minimal residual disease in acute lymphoblastic leukemia. *Hematol Am Soc Hematol Educ Program* 2010;**2010**:7–12.

19. Gokbuget N, Kneba M, Raff T, *et al.* Adults with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. *Blood* 2012;**120**:1868–76.

20. Holowiecki J, Krawczyk-Kulis M, Giebel S, *et al.* Status of minimal residual disease after induction predicts outcome in both standard and high-risk Ph-negative adult acute lymphoblastic leukaemia. The Polish Adult Leukemia Group ALL 4–2002 MRD Study. *Br J Haematol* 2008;**142**:227–37.

21. Krampera M, Vitale A, Vincenzi C, *et al.* Outcome prediction by immunophenotypic minimal residual disease detection in adult T-cell acute lymphoblastic leukaemia. *Br J Haematol* 2003;**120**:74–9.

22. Mortuza FY, Papaioannou M, Moreira IM, Coyle LA, Gameiro P, Gandini D, Prentice HG, Goldstone A, Hoffbrand AV, Foroni L. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. *J Clin Oncol* 2002;**20**:1094–104.

23. Raff T, Gokbuget N, Luschen S, *et al.* Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 trials. *Blood* 2007;**109**:910–5.

24. Vidriales MB, Perez JJ, Lopez-Berges MC, *et al.* Minimal residual disease in adolescent (older than 14 years) and adult acute lymphoblastic leukemias: early immunophenotypic evaluation has high clinical value. *Blood* 2003;**101**:4695–700.

25. Moorman AV, Richards SM, Robinson HM, Strefford JC, Gibson BE, Kinsey SE, Eden TO, Vora AJ, Mitchell CD, Harrison CJ. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). *Blood* 2007;**109**:2327–30.

26. Pui CH, Evans WE. Acute lymphoblastic leukemia. *N Engl J Med* 1998;**339**:605–15.

27. Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, Vora A, Mitchell CD, Harrison CJ. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. *Lancet Oncol* 2010;**11**:429–38.

28. Moorman AV, Chilton L, Wilkinson J, Ensor HM, Bown N, Proctor SJ. A population-based cytogenetic study of adults with acute lymphoblastic leukemia. *Blood* 2010;**115**:206–14.

29. Strefford JC, van Delft FW, Robinson HM, *et al.* Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. *Proc Natl Acad Sci USA* 2006;**103**:8167–72.

30. Boissel N, Auclerc MF, Lheritier V, *et al.* Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. *Journal of Clinical Oncology* 2003;**21**:774–80.

31. de Bont JM, Holt B, Dekker AW, van der Does-van den Berg A, Sonneveld P, Pieters R. Significant difference in outcome for adolescents with acute lymphoblastic leukemia treated on pediatric vs adult protocols in the Netherlands. *Leukemia* 2004;**18**:2032–5.

32. Larson S, Stock W. Progress in the treatment of adults with acute lymphoblastic leukemia. *Curr Opin Hematol* 2008;**15**:400–7.

33. Schiffer CA. Differences in outcome in adolescents with acute lymphoblastic leukemia: a consequence of better regimens? Better doctors? Both? *J Clin Oncol* 2003;**21**:760–1.