TABLE II. Patients Characteristics

Therapy groups	G1	G2	G3	G4	Total (%)
No. of patients	17	103	111	90	321
Sex					
Male	12	72	90	71	245 (76)
Female	5	31	21	19	76 (24)
Age					
0–4	2	12	18	16	48 (15)
5–9	3	45	42	39	129 (40)
10–14	8	42	42	27	119 (37)
15–	4	4	9	8	25 (8)
Histology					
BL/BLL/B-ALL	5	33	62	80	180 (56)
DLBCL	12	58	26	5	101 (31.4)
MLBCL	0	0	2	0	2 (0.6)
Others	0	12	21	5	38 (12)
Primary sites					
Thorax	5	30	7	1	43
Head & neck	5	39	12	2	58
Peripheral lymph nodes	0	3	3	0	6
Abdomen	7	29	75	11	122
Mediastinum	0	0	8	0	8
B-ALL	0	0	0	73	73
CNS	0	0	0	2	2
Other tumor site	0	2	5	0	7
Not specified	0	0	1	1	2
BM involvement	0	0	22	80	102 (32)
CNS involvement	0	0	0	38	38 (12)

BL, Burkitt lymphoma; BLL, Burkitt-like lymphoma; B-ALL, Burkitt leukemia; DLBCL, diffuse large B-cell lymphoma, MLBCL, mediastinal large.

for patients (n = 10) with CNS involvement only (BM−, CNS+), and 75.0% ± 8.2% for patients (n = 28) with BM and CNS involvements (BM+/CNS+), ($P = 0.102$) (Fig. 3D). Outcome by treatment response to initial A courses were as follows: The 4-year OS and EFS for patients who achieved CR (n = 236) or CRu (n = 54) at the last evaluation time were 95.7% ± 1.6% and 93.5% ± 1.6%, and 96.1% ± 2.7% and 86.9% ± 4.6%, respectively, while the 4-year OS and EFS for patients (n = 13) who did not achieve CR/CRu was 69.2% ± 12.8% and 15.4% ± 10.1% ($P < 0.001$), respectively.

Treatment Failure Events

Forty patients experienced an event and 25 have died (Fig. 2). The cause of death was tumor progression in 14, infection in 7, stem cell transplantation-related death in 3, and pulmonary bleeding in 1. The 40 events consisted of 13 induction failures, 6 deaths, 20 relapses, and one second cancer. Of the13 patients (6 in Group 3 and 7 in Group 4) who failed the initial treatment, 4 patients in Group 3 received salvage therapy and achieved CRu. At the time of the last analysis, 8 patients (4 in Group 3 and 4 in Group 4) were alive without tumor. Death in remission occurred in 3/321 (1%) patients: two died of infection and one died of pulmonary bleeding. The longest duration before relapse from the start of therapy was 38.9 months in DLBCL and 13.6 months in Burkitt histology. Relapse sites were 10 in local, 6 in BM, 2 in BM+CNS, one in local + CNS, and one in CNS. All CNS relapse occurred in patients with BL, but not with DLBCL. Thus, isolated CNS failure was only one among 38 patients with CNS involvement. Of the 20 relapsed patients, 11 died and 9 survived without tumor. A second cancer occurred among the patients who failed the initial treatment: a 12-year-old male with BL developed a secondary malignancy with acute myeloid leukemia (FAB M5) 17 months after the initial diagnosis.

Toxicity

Acute toxicity of treatment courses (A and B) was evaluated by the scale of NCI-CTC version 2.0., and rates of acute toxicity Grade 3 among patients in Groups 2, 3, and 4 are shown in Supplemental Table I. Anemia and neutropenia were the most frequent hematological toxicities with grade III or IV in all groups. In particular, grade IV neutropenia occurred in almost all patients (>98%) during A courses. In nonhematologic toxicity, infection was the single most frequent occurring with grade III or IV at least once in 70% of patients although the rate of grade IV infection was very small (<1%). Stomatitis and hepatotoxicity were also frequent, occurring with grade III or IV at least once in 20–35% and 24–38% of patients, respectively. The rate of renal toxicity grade III was very low. Leukoencephalopathy was reported in two patients of Group 3, and their MRI findings disappeared within 2 months without neurological symptoms. The overall incidence of renal insufficiency associated with tumor lysis syndrome was 2 out of 96 (2%) in Group 4, and these required assisted renal support with continuous hemodiafiltration.

DISCUSSION

During the last two decades, the survival outcome of children with B-NHL has been markedly improved through consecutive

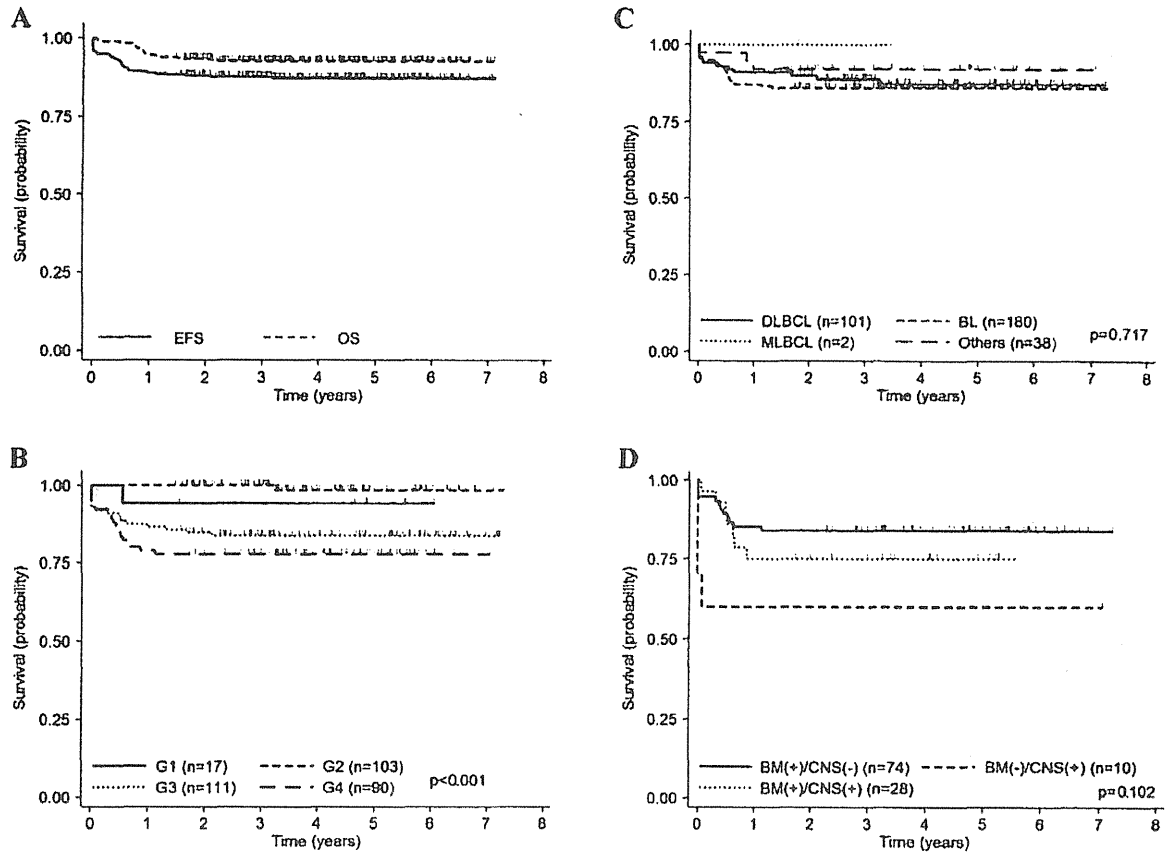

Fig. 3. Kaplan–Meier curves for OS and EFS of all patients (A). Kaplan–Meier curves for EFS according to treatment group (B), histology (C), and BM/CNS involvement (D).

clinical trials in large study groups, and the cure rate of childhood B-NHL has reached 90% [1–6]. In the present study, we showed an excellent survival outcome with 4-year OS 93% in children with B-NHL. In our study, the 4-year EFS 84% of Group 3 patients was considerably lower than the 4-year EFS 90% of intermediate risk group in the FAB/LMB96 study [5] or the 6-year EFS 88% of stage III patients in the BFM90 study [2], whereas, the 4-year EFS 78% of Group 4 patients compared favorably with the 4-year EFS 79% of high-risk group in the FAB/LMB96 study [5] and the 6-year EFS 74% of stage IV/B-ALL patients in the BFM90 study [2]. This outcome was obtained via the short-intensive chemotherapy regimen based on COPAD (CPM, VCR, PSL, and ADR) regimen plus the HDMTX of the lymphomas malin B (LMB) studies [3]. We omitted cranial irradiation for all patients, because recent studies have suggested the possibility of deleting radiotherapy in treating CNS diseases as well as CNS prophylaxis [2,3,5,9]. However, having no experience in administrating 8 g/m^2 HDMTX, we employed 5 g/m^2 HDMTX over 24 hour-infusion and not the 8 g/m^2 HDMTX over 4 hour-infusion in the LMB protocols for treating patients with CNS disease [3,5]. The treatment result for CNS disease was satisfactory, because CNS failure was only one of 38 patients with primary CNS disease in the present study.

This suggests that the 5 g/m^2 HDMTX over 24 hour-infusion is equally as effective to the CNS-positive disease as the aforementioned 8 g/m^2 HDMTX over 4 hours infusion, and reinforces the possibility that CNS irradiation could be omitted without jeopardizing the outcome of patients with CNS disease by using systemic and it MTX therapy [3,5,9].

The treatment of DLBCL as well as BL was another important focus of our study, because the incidence of DLBCL in childhood B-NHL is relatively more frequent than that of Western countries: the number of DLBCL was almost similar to that of BL (excluding B-ALL) in the present study and our recent national survey for childhood hematological malignancies has shown that the ratio of DLBCL to BL was 0.79 [14]. In our study, according to the strategy that DLBCL was treated by short-pulse chemotherapy as well as BL [15], we followed the same protocol, and achieved a favorable outcome of 4-year EFS with 87% for DLBCL which was not inferior to that of BL. This outcome can be partly explained by shared biological features, that is, that more than half of childhood DLBCL has the molecular subtypes of BL [16].

Several factors associated with poor outcome in the high-risk group in childhood B-NHL have been reported. Cairo et al. has shown a significantly inferior outcome (4-year EFS 61% ± 6%) of the subgroup of children with combined BM and CNS involvement at diagnosis as compared with children with BM or CNS only [5]. However, our results in Group 4 showed that the outcome (4-year EFS 75% ± 8%) of this subgroup with BM+/CNS+ was not significantly inferior than that of the subgroup with BM+ (83% ± 4%) or CNS+ (60% ± 1%). Failure to initial therapy is

also known to be a strong, unfavorable prognostic factor. Past studies in LMB 89/96 have shown that non-responders to pre-phase therapy (COP regimen) suffer a significantly inferior outcome as compared with responders or incomplete responders [3,5]. In our study, an appropriate evaluation of tumor regression just after pre-phase therapy was difficult for many patients, such that we compared the outcome according to response at the final evaluation time after two or three courses of therapy. These results showed that 4-year EFS of patients who did not achieve CR/CRu was only 15% ± 10%, which was as dismal as the outcome of poor-responders to COP regimen in the FAB/LMB 96 study [5]. To rescue the poor-responders in our study, we employed salvage therapy with high-dose Ara-C and VP16 to patients who did not achieve remission after 2 or 3 courses of therapy in Group 2 or 3, as in the BFM90 or FAB96 study [2,4]. As a result, 4 of 6 patients in Group 3 received salvage therapy and survived without tumor. This response rate was similar to that of FAB96 study, in which 10 out of 16 patients who received the second phase treatment intensification after the consolidation phase were alive. Thus, our results reconfirmed the efficacy of the salvage therapy.

Management of acute toxicity by short-pulse intensive chemotherapy is essential to successfully carry out the treatment protocol for childhood B-NHL. In our study, grade IV neutropenia occurred in almost all patients, but the rate of grade IV infection was quite low. Consequently, therapy-related death was less than 1% in all patients, and 2.1% in Group 4 patients. These results show the safety and feasibility of our treatment protocol. Anthracycline cardiotoxicity and secondary malignancy by alkylating agents are serious late events in pediatric cancer treatment [17,18]. To reduce the risk of cardiotoxicity, we employed THP-adriamycin (pirarubicin) instead of ADR. Pirarubicin is a derivative of ADR with reportedly less cardiotoxicity in adults [19–24]. Recently, we have reported that no significant cardiac dysfunction was detected in long-term survivors of children with acute lymphoblastic leukemia who received THP treatment [25–27]. In the present study, there were no patients with cardiac insufficiency or cardiac myopathy during the 7-year observation period. These results suggest that late-onset cardiotoxicity induced by pirarubicin is uncommon in childhood lymphoid malignancies, at least up to the cumulative dose of 240 mg/m^2. In our study, there was one male with a second cancer with acute myeloid leukemia, although the correlation between his second cancer and the protocol treatment is uncertain because he was resistant to the pre-phase followed by arbitrary treatment.

As shown above, chemotherapy-related toxicity of our protocol treatment was within acceptable range. However, a 6-course treatment for Group 3 seemed to be more intensive as compared with a 4-course treatment for intermediate risk group in the FAB96 study [4]. In order to reduce the total dose of cytotoxic drugs without impairing the survival outcome, new approaches including targeted monoclonal antibody therapy in combination with chemotherapy [28,29], are needed for children with an advanced or resistant disease in coming studies.

In conclusion, our nationwide study resulted in a cure rate above 90% with <1% toxic death in childhood B-NHL.

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ORIGINAL ARTICLE

Children and adolescents with follicular lymphoma have an excellent prognosis with either limited chemotherapy or with a "watch and wait" strategy after complete resection

Andishe Attarbaschi · Auke Beishuizen · Georg Mann · Angelo Rosolen · Tetsuya Mori · Anne Uyttebroeck · Felix Niggli · Monika Csoka · Zdenka Krenova · Karin Mellgren · Edita Kabickova · Alan KS Chiang · Alfred Reiter · Denise Williams · Birgit Burkhardt · on behalf of the European Intergroup for Childhood Non-Hodgkin Lymphoma (EICNHL) and the international Berlin-Frankfurt-Münster (i-BFM) Study Group

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Abstract Data on clinical features and outcome in pediatric follicular lymphoma (pFL) are scarce. The aim of this retrospective study including 13 EICNHL and/or i-BFM study group members was to assess clinical characteristics and course in a series of 63 pFL patients. pFL was found to be associated with male gender (3:1), older age (72 % ≥10 years old), low serum LDH levels (<500 U/l in 75 %), grade 3 histology (in 88 %), and limited disease (87 % stage I/II disease), mostly involving the peripheral lymph nodes. Forty-four out of sixty-three patients received any polychemotherapy and 1/63 rituximab only, while 17/63 underwent a "watch and wait" strategy. Of 36 stage I patients, 30 had complete resections. Only one patient relapsed; 2-year event-free survival and overall survival were 94±5 and 100 %, respectively, after a median follow-up of 2.2 years. Conclusively, treatment outcome in pFL

A. Attarbaschi (✉) · G. Mann
Pediatric Hematology and Oncology, St. Anna Children's Hospital, Kinderspitalgasse 6,
1090 Vienna, Austria
e-mail: andishe.attarbaschi@stanna.at

A. Beishuizen
Pediatric Hematology and Oncology, Erasmus MC—Sophia Children's Hospital, Rotterdam, The Netherlands

A. Rosolen
Pediatric Hematology and Oncology, University of Padova, Padova, Italy

T. Mori
National Hospital Organization Nagoya Medical Center, Aichi, Japan

A. Uyttebroeck
Pediatric Hematology and Oncology, University of Leuven, Leuven, Belgium

F. Niggli
Pediatric Hematology and Oncology, University Hospital, Zurich, Switzerland

M. Csoka
Pediatric Hematology and Oncology, Semmelweis University, Budapest, Hungary

Z. Krenova
Pediatric Hematology and Oncology, University Hospital, Brno, Czech Republic

K. Mellgren
Pediatric Hematology and Oncology, The Queen Silvia's Hospital for Children and Adolescents, University of Gothenburg, Gothenburg, Sweden

E. Kabickova
Pediatric Hematology and Oncology, University Hospital, Prague, Czech Republic

A. K. Chiang
Department of Pediatrics and Adolescent Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong

A. Reiter · B. Burkhardt
Pediatric Hematology and Oncology, Justus Liebig University, Giessen, Germany

D. Williams
Pediatric Hematology and Oncology, Cambridge University Hospitals Foundation Trust, Addenbrooke's Hospital, Cambridge, UK

B. Burkhardt
Pediatric Hematology and Oncology, University of Münster, Münster, Germany

seems to be excellent with risk-adapted chemotherapy or after complete resection and an observational strategy only.

Keywords Follicular lymphoma · Complete resection · "Watch and wait" · Outcome

Introduction

While follicular lymphoma (FL) accounts for 25 % of non-Hodgkin's lymphomas (NHL) in adulthood, it rarely occurs in children and adolescents (<2 % of cases) [1–4]. FL is recognized as a unique histopathological entity in the pediatric age group, with a high proportion, having grade 3 morphology, and no *BCL2*-rearrangement [1, 3, 5]. Moreover, while most adult patients present with disseminated disease at initial diagnosis, children usually present with localized disease often confined to the peripheral lymph nodes only [1, 3, 6, 7]. Optimal treatment of pediatric FL (pFL) has not yet been defined, and therapeutic strategies differ considerably with some groups applying intensive B cell NHL-type chemotherapy according to the stage of disease, others relying on CHOP-like cycles±rituximab and others favoring a "watch and wait" strategy after complete resection for at least *BCL2*–negative pFL [3, 6–12]. Regardless of the type of therapy, cure rates approach 90 % [3, 6, 8–10, 12]. Nevertheless, systematic data are scarce regarding clinical, biological, and outcome data in children and adolescents with FL. Thus, the two largest consortia in childhood NHL, the European Intergroup for Childhood NHL (EICNHL) and the international Berlin–Frankfurt–Münster (i-BFM) group, designed a retrospective multinational study on this rare B cell NHL. Herein, we report on the characteristics and outcome of 63 patients with pFL included in this analysis.

Patients and methods

Between May and December 2011, we performed an international survey of pFL, including only patients with nationally centrally reviewed histopathology from 13 EICNHL and/or i-BFM study group members. The survey included questions on demographics and disease (age, gender, sites of involvement, stage of disease, pretherapeutic lactate dehydrogenase (LDH) level) as well as on treatment (surgery, chemotherapy, radiotherapy) and outcome (date of remission, relapse, death, last follow-up). After 2000, a total of 63 children and adolescents up to 18 years old diagnosed with pFL were identified in the respective countries. The diagnosis was based on morphological and immunophenotypic criteria according to the World Health Organization classification [13]. Staging procedures as well as therapy protocols applied to the patients are described in detail elsewhere [3, 11, 14–19]. Most, if not all, patients were treated according to national treatment guidelines. All patients were treated, with informed consent from the patients, patient's parents, or legal guardians. Studies were conducted in accordance with the Declaration of Helsinki, and approval was delivered by the ethic committees. Event-free survival (EFS) and overall survival (OS) were estimated with Kaplan–Meier curves.

Results and discussion

Among the 63 patients, the male to female ratio was 3:1, and median age was 13.0 years (range 1.4–17.1 years), with 45/63 patients (72 %) ≥10 years old. The median pretherapeutic serum LDH level was 252 U/l (range 93–550 U/l), with 47/63 patients (75 %) having levels <500 U/l. Thirty-six out of sixty-three (57 %) had stage I (30 (83 %) with initial complete resection), 19 (30 %) stage II (2 (11 %) with initial complete resection), six (10 %) stage III, and two (3 %) children had stage IV disease, according to the St. Jude staging system, resulting in 54/63 patients (87 %) with limited stage I/II disease [19]. Details on patient characteristics and sites of involvement are summarized in Table 1, showing that 50/63 patients (79 %) had peripheral lymph node involvement. Histopathological grading was available in 48/63 patients (76 %), demonstrating grade 1 or 2 morphology in 6/48 (12.5 %) and grade 3 morphology in 42/48 patients (87.5 %). Nine out of forty-two patients (21 %) with grade 3 pFL had components of diffuse large B cell lymphoma (DLBCL).

Forty-four out of sixty-three patients (70 %) received any polychemotherapy and one (2 %) rituximab only, while 17 (26 %) underwent a "watch and wait" strategy (all with initial complete resection) (Table 1). In one patient (2 %), the type of therapy received could not be retrieved. Of the 38/44 patients with available information, all but two patients received low or intermediate risk B cell NHL-type therapy (Table 1). Only 1/63 patients (2 %) relapsed (after "watch and wait"), and none of the patients died from the disease itself or therapy-related toxicity. The 2-year EFS and OS rates were 94±5 and 100 % (Fig. 1), respectively, after a median follow-up of 2.2 years (range 0.19–8.71 years).

To our knowledge, this report including 63 patients with centrally reviewed pFL covering a time period >10 years represents by far the largest series of pFL in childhood and adolescence reported to date. Although the analysis has been conducted retrospectively, was not population-based, and patients were not treated according to a common protocol or strategy, it allows several insights into the clinical presentation and outcome of pFL patients and thus may have important implications on the future management of this

Table 1 Clinical, laboratory, and treatment characteristics as well as outcome of the 63 patients with pediatric follicular lymphoma

Variable	No. of pts.
Gender	
Male	47 (75 %)
Female	16 (25 %)
Age (y)	
Median	13.0
Range	1.4–17.7
<10	18 (28 %)
≥10–15	25 (40 %)
≥15	20 (32 %)
sLDH level (U/l)	
Median	252
Range	93–550
<500	47 (75 %)
≥500	5 (8 %)
n.a.	11 (17 %)
Stage of disease	
Stage I	36 (57 %)
Stage II	19 (30 %)
Stage III	6 (10 %)
Stage IV	2 (3 %)
Histological grading	
Grade 1	4 (6 %)
Grade 2	1 (2 %)
Grade 3[a]	27 (43 %)
Grade 1+2	1 (2 %)
Grade 1+3a	1 (2 %)
Grade 1+2+3a+MZL	1 (2 %)
Grade 2+3a	2 (3 %)
Grade 3+DLBCL[b]	9 (14 %)
Grade 3a+MZL	2 (3 %)
n.a.	15 (24 %)
Sites of involvement[c]	
Peripheral lymph nodes*	50 (79 %)
Head and neck (extranodal)	1 (2 %)
Tonsils	4 (6 %)
Ear–nose–throat	4 (6 %)
Mediastinum	0
Abdomen	9 (14 %)
Bone marrow	2 (3 %)
Central nervous system	0
Testis	2 (3 %)
Skin	1 (2 %)
Bone	1 (2 %)
Resection status	
Incomplete/biopsy	26 (41 %)
Complete	32 (51 %)
n.a.	5 (8 %)
Treatment	
Chemotherapy[e]	44 (70 %)
Rituximab only	1 (2 %)
"Watch and wait"	17 (26 %)
n.a.	1 (2 %)
Complete resection	
"Watch and wait"	17 (53 %)
Chemotherapy	15 (47 %)
Resection acc. to stage	
Stage I	36
Stage I-R	30 (83 %)
Stage I-NR	4 (11 %)
Stage I-n.a.	2 (6 %)
Stage II	19
Stage II-R	2 (10 %)
Stage II-NR	14 (74 %)
Stage II-n.a.	3 (16 %)
Stage III/IV-NR	8 (100 %)
Radiotherapy	
Yes	1 (2 %)
No	61 (96 %)
n.a.	1 (2 %)
Outcome	
Relapse	1 (2 %)
Death	0
2-year EFS	94±5 %
2-year OS	100 %
Follow-up (y)	
Median	2.2
Range	0.2–8.7
Lost to follow-up	1 (2 %)[f]

No. of pts number of patients, *y* years, *sLDH* serum lactate dehydrogenase, *n.a.* not available, *MCL*, marginal zone lymphoma, *DLBCL* diffuse large B cell lymphoma, *acc.* according, *R* complete resection, *NR* no complete resection, *CCR* complete continuous remission, *EFS* event-free survival, *OS* overall survival

[a] 13/27 with grade 3a, 10/27 with grade 3b, and 3/27 patients with no information on the 3a/3b variant; [b] 3/9 with grade 3a and 6/9 patients with grade 3b morphology, [c] 27/63 patients suffered from stage II, III, or IV disease and thus had >1 site of involvement. [d] corresponding to cervical (submandibular), supra- and infraclavicular, pre- and retroauricular, nuchal, parotical, axillary, and inguinal lymph node regions, [e] according to protocols of the NHL-BFM (n=27), AIEOP (n=3), LMB (n=2), JACLS (n=5), and UKCCSG (n=1) studies; CHOP (n=5), CVP (n=1). [f] This patient was lost to follow-up immediately after the primary operation

indolent disease. Our data convincingly show that pFL is usually associated with male gender (3:1), older age (40 % 10–15 years, 32 % ≥15 years old), low serum LDH levels (<500 U/l in 75 %), and limited disease (87 % with stage I/II

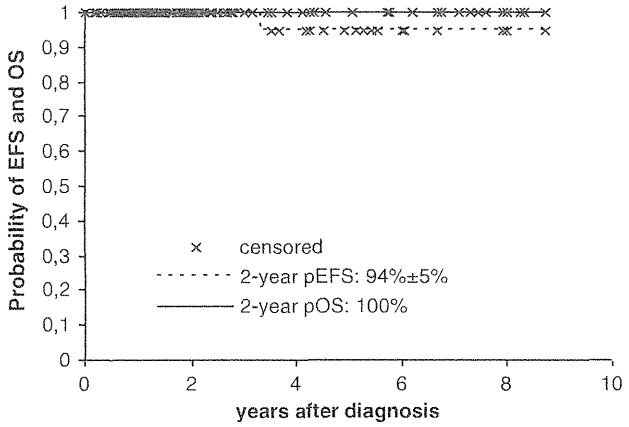

Fig. 1 Two-year event-free and overall survival of the 63 patients with pediatric follicular lymphoma

disease), mostly involving the peripheral lymph nodes. However, as we identified stage III/IV patients, initial diagnostic work-up should always follow the St. Jude staging system [19]. Due to its rarity, only few case reports and series on pFL have been published so far with patient numbers ranging from 4–25 [3, 6, 8–10, 12]. Most of the reports demonstrated similar findings concerning the initial clinical and laboratory features of pFL [1, 3, 6, 8–10, 12].

Nonetheless, we demonstrated that in contrast to FL in adults which is usually of low-grade morphology and not curable with diverse treatment approaches, pFL is frequently associated with grade 3 morphology and has a very good outcome after limited chemotherapy or complete resection followed by a "watch and wait" strategy [3, 11, 20]. Chemotherapy was performed according to stage-adapted protocols of the NHL-BFM (n=27), AIEOP (n=3), LMB (n=2), JACLS (n=5), and UKCCSG (n=1) studies and with CHOP (n=5) and CVP (n=1) cycles, respectively [6, 15–18, 21, 22].

Importantly, neither higher histological grading nor initial components of DLBCL were associated with an unfavorable prognosis. In addition, of the 32 patients with initial complete resection (including 30/36 stage I patients), 17 (53 %) children had no further treatment with only one relapse (local), suggesting no systemic disease in localized pFL. The excellent overall outcome of our cohort of FL patients is comparable to the results published in the literature, showing that pediatric stage-adapted B cell NHL-type chemotherapy and CHOP-like cycles±rituximab are effective in (in)completely resectable disease [1, 3, 6, 8–10, 12, 22]. However, the exact role of complete resection and observation has not been validated until yet. Thus, future clinical trials should aim to establish the least amount of effective (chemo) therapy necessary for cure of pFL. As almost all cycles of chemotherapy used for pediatric B cell NHL include anthracyclines, alkylating agents, and intrathecal therapy, low intensity chemotherapy for pFL should be ideally free of the latter components usually carrying the risk for acute and long-term toxicity [16–18, 23]. A recent study in pediatric early-stage nodular lymphocyte predominant Hodgkin's lymphoma may serve as a paradigm, as it has shown that low intensity chemotherapy is successful in noncompletely resectable disease, while more toxic treatment blocks applied for classic Hodgkin's lymphoma can be reserved for relapse [24].

Notably, there are several limitations when analyzing data from a multinational retrospective survey on a very rare lymphoma subtype, all of which necessitate further evaluation in well-defined prospective trials. As such, we were unable to report on genetic studies, minimal residual disease screening, and in particular on how and why the decision was taken by the responsible physicians to follow a "watch and wait strategy" or chemotherapy in completely resected disease.

Nevertheless, based on the data gained from our unique survey on pFL, we concluded that in the case of complete resections in carefully evaluated stage I patients a "watch and wait" strategy might be possible. However, we suggest that patients are only candidates for complete surgical resection if the operation can be performed easily and safely, and, most importantly, without any functional impairment. In all other patients, initial surgery should include the least invasive procedure to establish the diagnosis followed by limited chemotherapy. Given the difficulties in differentiating pFL from reactive lymphadenopathy, evaluation by an experienced hematopathologist is highly recommended before starting any therapy [13]. As children with nonresectable pFL had an excellent outcome with multidrug chemotherapy, which is associated with acute and long-term toxicity, multinational controlled trials have to be performed, taking genetics (*BCL2*, *BCL6*, *IGH*, *C-MYC*) into account, to clearly establish not only that no chemotherapy is a safe approach in stage I patients with complete resection, but low intensity chemotherapy ± monoclonal antibodies is sufficient for patients with noncompletely resectable disease [7, 21–23, 25, 26].

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Conflicts of interest The authors declare no competing financial interests.

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BRIEF REPORT

Central Nervous System Involvement in Anaplastic Large Cell Lymphoma in Childhood: Results From a Multicentre European and Japanese Study

Denise Williams, MB Bch,[1]* Tetsuya Mori, MD PhD,[2] Alfred Reiter, MD,[3] Wilhelm Woessman, MD,[3] Angelo Rosolen, MD,[4] Grazyna Wrobel, MD,[5] Jozsef Zsiros, MD PhD,[6] Anne Uyttebroeck, MD PhD,[7] Ildiko Marky, MD,[8] Marie-Cécile Le Deley, MD PhD,[9,10] Laurence Brugières, MD,[11] and for the European Intergroup for Childhood Non-Hodgkin Lymphoma, the Japanese Pediatric Leukemia/Lymphoma Study Group

In an international study of systemic childhood ALCL, 12/463 patients had CNS involvement, three of which had isolated CNS disease. Comparative analysis of CNS positive and negative patients showed no difference in ALK positivity, immunophenotype, presence of B symptoms or other sites of disease. The lymphohistiocytic variant was over represented in the CNS positive group (36% vs. 5%). With multi-agent chemotherapy, including high dose methotrexate, Ara-C and intrathecal treatment, the event free and overall survival of the CNS positive group at 5 years were 50% (95%CI, 25–75%) and 74% (45–91%), respectively with a median follow up of 4.1 years. Pediatr Blood Cancer 2013;60:E118–E121. © 2013 Wiley Periodicals, Inc.

Key words: anaplastic large cell lymphoma; childhood; CNS disease; lymphohistiocytic; non-Hodgkin lymphoma

INTRODUCTION

Central nervous system (CNS) involvement in anaplastic large cell lymphoma (ALCL) is rare at diagnosis and relapse and there are few published data. Most publications report collectively on CNS disease in childhood NHL, of which ALCL constitutes small numbers [1], or are single case reports [2,3]. In 1999, the European Intergroup for Childhood NHL (EICNHL) designed a prospective multinational study for treatment of childhood ALCL (ALCL99). We report the incidence, clinical pattern and outcome of CNS disease in this study.

PATIENTS AND METHODS

Between 1999 and 2006, 463 children with systemic ALCL from 10 study groups and 12 countries were registered with informed consent on the ALCL99 study. The study involved registration of all children with ALCL and a randomised treatment trial according to risk factors defined by the previously reported prognostic factor study [4]. Patients with CNS disease were registered but were excluded from the treatment trial.

ALCL diagnosis was based on histopathology and immunohistochemistry according to the revised European American classification of lymphoid malignancies. Pathological review was performed in 96% of registered patients, including all patients with CNS involvement [5].

Staging was performed as described within the protocol [6,7]. A cerebrospinal fluid (CSF) examination was performed at diagnosis in all patients and cranial imaging, CT or MRI, recommended in patients with neurological symptoms. CNS involvement was defined as the presence of lymphoma cells in the CSF, demonstration of the t(2;5) translocation in the CSF, presence of a cranial nerve palsy (CNP) not explained by a lesion outside the CNS or an intracerebral (ICM) or spinal lesion on imaging.

Patients with CNS disease were treated according to the preference of the treating national group with protocols designed for B-NHL with CNS involvement, either the Berlin-Frankfurt-Münster (BFM) group BFM 90 protocol branch K3 [8] or the LMB 96/2001 of the French Society of Paediatric Oncology (SFCE) [9,10]. Some patients, though ineligible, were treated on the ALCL 99 protocol [6]. Cranial radiotherapy was recommended depending on age and response to chemotherapy.

Clinical and pathological characteristics were compared between the CNS-positive and CNS-negative patients using Fisher's exact tests. Event-free and overall survival were estimated with Kaplan–Meier curves and comparisons performed with log-rank tests.

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[1]Department of Pediatric Oncology, Cambridge University Hospital NHS Trust, Cambridge, United Kingdom; [2]Department of Paediatrics, National Center for Child Health and Development, Tokyo, Japan; [3]Non-Hodgkin Lymphoma–Berlin-Frankfurt-Muenster Study Centre, Department of Pediatric Hematology and Oncology, Justus-Liebig-University, Giessen, Germany; [4]Department of Pediatrics, University Hospital, Padova, Italy; [5]Department of Bone Marrow Transplantation, Children Oncology and Hematology, Medical University, Wroclaw, Poland; [6]Department of Pediatric Oncology, Emma Kinderziekenhuis, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [7]Department of Pediatric Hematology/Oncology, University Hospital, Leuven, Belgium; [8]Department of Pediatrics, Göteborg University, Göteborg, Sweden; [9]Biostatistics and Epidemiology Unit, Institut Gustave-Roussy, Villejuif, France; [10]Paris-Sud University, Le Kremlin-Bicetre, France; [11]Department of Pediatric Oncology, Institut Gustave-Roussy, Villejuif, France

Authorship contributions: L.B., M.L.D. and A.R. (Reiter), D.W. and A. Rosolen designed and planned the study; D.W., T.M., L.B. and M.L.D. wrote the manuscript; M.L.D. was in charge of data pooling, data checking and statistical analysis; all other authors (A.U., I.M., W.W., G. W., J.Z.) as well as L.B., A.R. (Reiter) and D.W. were principal or co-investigators in their study groups and institutions, coordinated the study in their countries, provided study materials and recruited patients. All authors read and approved the final version of the manuscript.

Conflict of interest: Nothing to declare.

*Correspondence to: Dr. Denise Williams, Department of Pediatric Oncology, Cambridge University Hospital NHS Trust, Cambridge CB2 0QQ, UK. E-mail: denise.williams@addenbrookes.nhs.uk

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TABLE I. Tweleve Patients With Anaplastic Large Cell Lymphoma With Central Nervous System (CNS) Disease: Pattern of CNS Disease, Sites of Disease, Treatment and Response to Treatment

Patient	CNS disease CNP/ICM/LCCSF	Other sites of disease	Histologic subtype/ immunophenotype	Chemotherapy protocol	RT Y/N dose	CR	Relapse	Site of disease at relapse	Outcome: Alive (A)/dead (D) median follow up of 4.1 yrs (range 2.3–7.4)
1	LCCSF	Nodes, skin	LH/T cell	BFM NHL 90 K3	N but planned	Y	Y	Skin, spleen, nodes	D (TRM after further relapse)
2	LCCSF	Nodes, liver, skin, BM	LH/T cell	ALCL 99	N	N			D
3	LCCSF/ICM	None	Unclassified/T cell	BFM NHL 90 K3	Y 24Gy	Y			A
4	CNP/ICM	None	LH/T cell	BFM NHL 90 K3	Y 24Gy	Y			A
5	ICM	Nodes, mediastinum, lung, liver, spleen	LH/T cell	ALCL 99	N	Y			A
6	LCCSF	Nodes, sinuses, ENT	Common type/null cell	BFM NHL 90 K3	N but planned	N			A
7	ICM	Lung	Mixed/T cell	LMB96 Group C	N but planned	N			D
8	LCCSF	Nodes, skin	Common type/T cell	LMB96 Group C	N	Y	Y	CNS	D (TRM after further relapse)
9	CNP/ICM/LCCSF	Nodes, mediastinum, lung, liver, spleen	Common type/T cell	LMB96 Group C	Y 24 Gy	Y			A
10	CNP/ICM	Nodes, spleen, bone	Common type/T cell	LMB 2001 group C	Y 18 Gy	Y			A
11	LCCSF	Nodes, mediastinum, lung, liver, skin	Common type/T cell	LMB 2001 group C	N	Y			A
12	CNP/ICM	None	Common type/T cell	ALCL 99	N	Y	Y	CNS	A

CNS, central nervous system; CNP, cranial nerve palsy; ICM, intracerebral mass; LCCSF, lymphoma cells in the CSF; LH, lymphohistiocytic; RT, radiotherapy; CR, complete remission; TRM, treatment related mortality.

RESULTS

CNS involvement at diagnosis occurred in 12/463 (2.6%) patients registered in ALCL99 study (Table I). Lymphoma cells in the CSF alone were found in 5/12 (range 56–570 cells/mm^3), an ICM alone in 5/12, and 2/12 showed the presence of both. All four patients with a cranial nerve palsy also had an ICM. Isolated CNS involvement occurred in 3/12 patients, all with an ICM.

Comparative analysis of CNS positive and negative patients revealed no significant difference in the incidence of ALK positivity, immunophenotype, presence of B symptoms or other sites of disease. However, presence of a lymphohistiocytic (LH) histological component was significantly higher in the CNS positive patients, 4/11 (36%) compared to 23/423 (5%) of the CNS negative group ($P = 0.003$) (Table II). One CNS positive patient, though confirmed ALCL at review did not have a subtype allocated.

In patients with CNS and systemic disease, there appeared to be an association with other clinical risk factors; 8/9 patients having high risk features, skin, mediastinal, liver, spleen or lung involvement [4].

All 12 patients were treated with chemotherapy according to BFM 90 protocol branch K3 (n = 4) or LMB 96/2001 protocol (n = 5) and three with ALCL 99 protocol [6]: three did not achieve remission, two of them died and the third progressed but achieved complete remission (CR) with second line therapy (Table I). Among the 9/12 patients (75%) who achieved remission with initial therapy, six are alive in 1st CR and three relapsed systemically (one also in the CNS). Two died of toxicity during treatment after a further relapse and one patient achieved a second CR. Overall, 8/12 patients remain alive in first (n = 6) or second CR (n = 2). Four patients died, two of treatment related toxicity, and two of disease. Cranial radiotherapy (18–24 Gy) was planned in seven patients and performed in only four as three patients relapsed/progressed before radiotherapy. All 4 patients who received cranial irradiation as part of first line therapy remain in complete remission, whereas the five patients where the intention was to treat with chemotherapy alone, two patients relapsed in the CNS alone at 2 and 7 months off treatment. All four patients treated with radiotherapy had ICM's, two of them associated with systemic disease. With a median follow

TABLE II. Clinical and Pathological Characteristics of 463 Patients With Systemic ALCL, According to the CNS Involvement

	CNS negative n = 451		CNS positive n = 12		
Characteristics	N	%	N	%	*P*-value Fisher exact
Review					0.21
None	20	4	0	0	
National only	47	10	3	25	
International +/− National	384	85	9	75	
Sub-type (MD = 29a)					0.06
Common type	271	64	6	55	
Giant cell	8	2	0	0	
Small cell	24	6	0	0	
Lymphohistiocytic	11	3	3	27	
Hodgkin's like	9	2	0	0	
Mixed	98	23	2	18	
Other	2	<1	0	0	
Lymphohistiocytic component (MD = 29a)	23	5	4	36	0.003
ALK negative	20	4	1	8	0.43
Immunophenotype					>0.99
Null	55	12	1	8	
T-cell	396	88	11	92	
B-symptom	252	57	7	58	>0.99
Lymph nodes	392	87	7	58	0.02
Mediastinal involvement	208	46	4	33	0.56
Spleen involvement	77	17	5	42	0.04
Liver involvement	68	15	4	33	0.10
Lung involvement	91	20	5	42	0.08
Spleen, liver or lung involvement	149	33	7	58	0.12
Visceral involvement	215	48	7	58	0.56
Bone lesion	76	17	1	8	0.70
Skin lesion	85	19	3	25	0.71
High risk (involvement of spleen or liver or lung or mediastinal involvement or skin lesion)	283	63	8	67	>0.99
Bone marrow involvement	44	10	3	25	0.11
Ann Arbor staging (MD = 2)					$<10^{-3}$
St 1	42	9	0	0	
St 2	133	29	0	0	
St 3	137	30	0	0	
St 4	139	31	12	100	

[a]MD, missing data. The histological subtype was defined for 423 CNS negative and 12 CNS positive patients.

up of 4.1 years, the 5-year pEFS and OS of the CNS positive group were 50% (95%CI, 25–75%) and 74% (45–91%) (range 2.3–7.4), respectively.

DISCUSSION

The incidence of CNS disease reported here is similar to previous reports, which range from 0% to 22% [1,8,9,11–13]. This variation may be the result of small numbers, or varying definitions of CNS involvement [3]. The lymphohistiocytic variant seen in this study to be over represented in the CNS + ve group has been reported in case reports of patients with ALCL and CNS disease [2] and also to be associated with a poorer prognosis in systemic ALCL [9,14].

In patients with CNS and systemic disease, there appeared to be an association with other clinical risk factors with 8/9 patients having high risk features [4] supporting the findings of CNS disease in children with NHL being associated with high stage disease [1].

The inferior progression free survival of CNS positive as compared with CNS negative patients may be related to advanced stage disease, or poor risk histological variants, but appears to be an independent prognostic variable in this study [15].

Even though this series of ALCL with CNS involvement is the largest published to date, the number of patients is too small to allow comparison of the efficacy of chemotherapy regimens. Protocols including high dose MTX, Ara-C and intrathecal treatment seem to be effective in this population of patients since a CR could be obtained in 75% of the cases.

As cranial radiotherapy was only planned as part of initial treatment for 7/12 patients, its role is difficult to assess. It was performed more frequently in patients with ICM than in patients with tumour cells in the CSF. It is noteworthy that 5/7 patients with an ICM, who remain alive, had cranial radiotherapy as part of first or second line treatment and of 2/5 patients in whom radiotherapy was not planned suffered a CNS relapse. Though we cannot prove that radiotherapy is necessary to obtain long-term survival, these data support the recommendation of cranial radiotherapy for patients with ALCL and CNS involvement.

There are new promising approaches to the management of ALCL. Vinblastine has been shown to induce complete remission in patients with systemic ALCL even after multiple relapses [16] and is known to cross the blood–brain barrier. Its dual therapeutic effect with a direct killing of tumour cells and ability to boost host immunity [17] may be important in ALCL where the importance of an immune response to the ALK protein has been demonstrated [18]. Additionally, a number of new agents are being evaluated for systemic ALCL. These include brentuximab vedotin and the ALK inhibitor crizotinib [19]. Their role in CNS disease may be limited, crizotinib being shown to have poor CSF penetrance [20] but in combination with standard therapy may improve the outlook in this disease.

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Regular Article

LYMPHOID NEOPLASIA

Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group

Trudy D. Buitenkamp,[1] Shai Izraeli,[2] Martin Zimmermann,[3] Erik Forestier,[4] Nyla A. Heerema,[5] Marry M. van den Heuvel-Eibrink,[1] Rob Pieters,[1,6] Carin M. Korbijn,[6] Lewis B. Silverman,[7] Kjeld Schmiegelow,[8] Der-Cheng Liang,[9] Keizo Horibe,[10] Maurizio Arico,[11] Andrea Biondi,[12] Giuseppe Basso,[13] Karin R. Rabin,[14] Martin Schrappe,[15] Gunnar Cario,[15] Georg Mann,[16] Maria Morak,[16] Renate Panzer-Grümayer,[16] Veerle Mondelaers,[17] Tim Lammens,[17] Hélène Cavé,[18] Batia Stark,[19] Ithamar Ganmore,[2] Anthony V. Moorman,[20] Ajay Vora,[21] Stephen P. Hunger,[22] Ching-Hon Pui,[23] Charles G. Mullighan,[24] Atsushi Manabe,[25] Gabriele Escherich,[26] Jerzy R. Kowalczyk,[27] James A. Whitlock,[28] and C. Michel Zwaan[1]

[1]Pediatric Oncology/Hematology, Erasmus MC–Sophia Children's Hospital, Rotterdam, The Netherlands; [2]Department of Paediatric Haemato-Oncology, Edmond and Lily Safra Children's Hospital, Sheba Medical Centre, Tel-Hashomer, Ramat Gan, and Tel Aviv University Medical School, Tel Aviv, Israel; [3]Statistical Office of Berlin-Frankfurt-Münster Study Group, Pediatric Hematology/Oncology, Medical School Hannover, Hannover, Germany; [4]Department of Medical Biosciences, University of Umeå, Umeå, Sweden; [5]Department of Pathology, The Ohio State University, Columbus, OH; [6]Dutch Childhood Oncology Group, The Hague, The Netherlands; [7]Dana-Farber Cancer Institute, Boston, MA; [8]Pediatric and Adolescent Medicine, the Juliane Marie Centre, the University Hospital Rigshospitalet, Copenhagen, Denmark; [9]Pediatric Hematology-Oncology Division, Mackay Memorial Hospital, Taipei, Taiwan; [10]Clinical Research Center, National Hospital Organization, Nagoya Medical Center, Nagoya, Aichi, Japan; [11]Department Pediatric Hematology Oncology, Azienda Ospedaliero-Universitaria Meyer Children Hospital, Florence, Italy; [12]Departement of Pediatrics, Univerty of Milano-Bicocca, Ospedale S. Gerardo, Monza, Italy; [13]Pediatric Hemato-Oncology, Department of Pediatrics "Salus Pueri," University of Padua, Padova, Italy; [14]Division of Pediatric Hematology/Oncology, Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX; [15]Department of Pediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Germany; [16]Children's Cancer Research Institute, St. Anna Children's Hospital, University Medical School Vienna, Vienna, Austria; [17]Department of Pediatric Hemato-Oncology, Ghent University Hospital, Belgium; [18]Assistance Publique, Hôpitaux de Paris, Hôpital Robert Debré, Département de Génétique Université Paris-Denis Diderot, Paris, France; [19]Center of Pediatric Hematology/Oncology, Schneider Children's Medical Center of Israel, Petah Tiqwa, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [20]Leukaemia Research Cytogenetics Group, Northern Institute for Cancer Research, Newcastle University, Newcastle-upon-Tyne, United Kingdom; [21]Department of Haematology, Sheffield Children's Hospital, Sheffield, United Kingdom; [22]Department of Pediatrics, University of Colorado School of Medicine and Children's Hospital, Aurora, CO; [23]Department of Oncology and [24]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN; [25]Department of Pediatrics, St. Luke's International Hospital, Tokyo, Japan; [26]Clinic of Pediatric Hematology and Oncology, University Medical Center Hamburg–Eppendorf, Hamburg, Germany; [27]Department of Children's Hematology and Oncology, Medical University, Lublin, Poland; and [28]Division of Haematology/Oncology, the Hospital for Sick Children, University of Toronto, Toronto, Canada

Key Points

- Although the risk of ALL relapse is significantly higher in children with DS, good-prognosis subgroups have been identified.
- Patients with DS-ALL have higher treatment-related mortality throughout the treatment period independent of the therapeutic regimen.

Children with Down syndrome (DS) have an increased risk of B-cell precursor (BCP) acute lymphoblastic leukemia (ALL). The prognostic factors and outcome of DS-ALL patients treated in contemporary protocols are uncertain. We studied 653 DS-ALL patients enrolled in 16 international trials from 1995 to 2004. Non-DS BCP-ALL patients from the Dutch Child Oncology Group and Berlin-Frankfurt-Münster were reference cohorts. DS-ALL patients had a higher 8-year cumulative incidence of relapse (26% ± 2% vs 15% ± 1%, $P < .001$) and 2-year treatment-related mortality (TRM) (7% ± 1% vs 2.0% ± <1%, $P < .0001$) than non-DS patients, resulting in lower 8-year event-free survival (EFS) (64% ± 2% vs 81% ± 2%, $P < .0001$) and overall survival (74% ± 2% vs 89% ± 1%, $P < .0001$). Independent favorable prognostic factors include age <6 years (hazard ratio [HR] = 0.58, $P = .002$), white blood cell (WBC) count $<10 \times 10^9$/L (HR = 0.60, $P = .005$), and *ETV6-RUNX1* (HR = 0.14, $P = .006$) for EFS and age (HR = 0.48, $P < .001$), *ETV6-RUNX1* (HR = 0.1, $P = .016$) and high hyperdiploidy (HeH) (HR = 0.29, $P = .04$) for relapse-free survival. TRM was the major cause of death in *ETV6-RUNX1* and HeH DS-ALLs. Thus, while relapse is the main contributor to poorer survival in DS-ALL, infection-associated TRM was increased in all protocol elements, unrelated to treatment phase or regimen. Future strategies to improve outcome in DS-ALL should include improved supportive care throughout therapy and reduction of therapy in newly identified good-prognosis subgroups. (*Blood*. 2014; 123(1):70-77)

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S.I., J.A.W., and C.M.Z. contributed equally to this study.

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Introduction

Children with Down syndrome (DS) are predisposed to develop acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL),[1] which are characterized by unique biological features in comparison with those of non–DS-ALL.[2-4]

Children with DS-ALL have an inferior outcome compared with non-DS patients because of both higher treatment-related mortality (TRM) and a higher relapse rate.[5-9] Because attempts to decrease TRM by reducing treatment intensity may contribute to the increased risk of relapse in DS-ALL, it is important to determine whether the risk for TRM is related to a specific treatment phase or chemotherapeutic agent.[8-10] Small series suggest that DS-ALL patients have an increased risk of mucositis from methotrexate (MTX), myelosuppression from anthracyclines, and hyperglycemia from glucocorticoids.[10-16]

Acquired leukemic cell genetic abnormalities have important prognostic significance in non-DS childhood ALL.[17] However, the impact of these abnormalities on treatment outcome in DS-ALL is unknown, because all published series lack a sufficient sample size to draw clear conclusions. Even the prognostic significance of well-known good prognostic factors in non–DS-ALL such as t(12;21)(p13;q22) (*ETV6-RUNX1*), high hyperdiploidy (HeH), and trisomies 4 and 10 is uncertain in DS-ALL, as is the significance of unfavorable translocations such as t(9;22)(q34;q11) (*BCR-ABL1*) and t(4;11)(q21;q23) (*MLL-AF4*).[2] Of interest, these prognostic genetic features have a lower frequency in DS-ALL.[2,7,18,19]

Recently, genetic abnormalities such as *JAK2* mutations[20] and *CRLF2* rearrangements have been identified in both DS and non–DS-ALL.[3,4,20-27] Activating *JAK2* R683 mutations were found in ~18% of DS-ALL patients.[20,24] Rearrangements of *CRLF2* occurred in ~60% of DS-ALL patients and in fewer than 10% of non–DS-ALL patients.[3,4,23] In almost all instances, *JAK2* (or rarely *JAK1* or *IL7R*) mutations were associated with *CRLF2* gene rearrangements, suggesting a model by which *CRLF2* overexpression results in JAK-STAT activation and proliferation of the leukemic clone.[3] Thus far, *CRLF2* gene rearrangements lack prognostic relevance in DS-ALL, although all series were small.[3,4,21,27]

The small size of most studies in DS-ALL patients has precluded definitive answers to the issues raised above. Hence, we undertook a large retrospective study of DS-ALL within the International ALL "Ponte di Legno" Working Group to study clinically relevant outcome parameters, the prognostic relevance of well-established and novel (cyto)genetic aberrations in ALL, and causes of treatment failure, thereby allowing a sufficient sample size to draw meaningful conclusions, despite the caveat of heterogeneity in treatment over time and between different study groups.[28]

Patients and methods

Patients

Patients eligible for this study were enrolled in various national or collaborative group clinical trials between January 1, 1995, and December 31, 2004, were ≤18 years at diagnosis and were treated with curative intent. The institutional review boards of each participating center approved treatment protocols according to the local law and guidelines. Informed consent was obtained in accordance with the Declaration of Helsinki. Participating study groups and their number of patients are detailed in supplemental Table 1 (available on the *Blood* Web site). A predefined set of data were collected, consisting of clinical data obtained at diagnosis and treatment and cytogenetic and molecular data (supplemental Table 2).

DS-ALL patients were treated according to standard ALL treatment protocols, but modifications of the standard protocol did occur. None of the protocols provided specific supportive care measures for DS-ALL children. In total, 42.3% (n = 276) DS-ALL patients received a reduced dose of chemotherapy. Most of these dose reductions (79%) were planned prior to the administration of specific courses of chemotherapy and gradually increased by observed clinical toxicity. Modifications for MTX consisted of dose reductions of high-dose MTX, varying from 10% to 75% of the maximum dose, and intensified leucovorin rescue. DS-ALL patients enrolled in EORTC 58951 protocols from September 2002 (n = 7) received 0.5 g/m^2 of MTX instead of 5 g/m^2. In addition, patients treated on protocol POG 9405 (n = 10) started with 50% of the total dose of daunorubicin, cytarabine, teniposide, histone deacetylase, and Peg-asparaginase, which was successively increased or reduced depending on toxicity. Supplemental Table 3 provides an overview of the main chemotherapeutic agents of treatment protocols used by the various study groups.

Data on either *JAK2* R683 mutations and/or *CRLF2* gene rearrangements were available from a subset of patients (n = 182) included in this study. There were no statistical differences between patients with and without available data. Some of these data have been previously reported.[20,24,25] However, several study groups contributed new unpublished data.

Non–DS-ALL reference cohort

For comparison, population-based B-cell precursor (BCP) ALL reference cohorts from the Dutch Child Oncology Group (DCOG) and the ALL Berlin-Frankfurt-Münster (BFM) study group, from exactly the same time period as the DS patients (January 1, 1995, and December 31, 2004), were added. The DCOG data set consisted of 827 non-DS BCP-ALL patients enrolled in 3 DCOG ALL treatment protocols (ALL8, ALL9, and ALL10). The BFM data set consisted of 3618 non-DS BCP-ALL patients enrolled in 2 BFM treatment protocols (BFM-95 and BFM-2000) in Germany and Austria. Details of these protocols have been reported elsewhere, except for protocol ALL10, which is ongoing.[29,30]

There were no significant differences in outcome estimates or in the distribution of cytogenetic subgroups between the DCOG and BFM data sets (data not shown) or when compared with reported data from other participating groups.[31-38] The DCOG and BFM non-DS data sets were merged for statistical analysis.

Cytogenetic analysis

Genetic abnormalities were determined by G-, Q-, or R-banded karyotyping, fluorescence in situ hybridization (FISH), or reverse-transcription polymerase chain reaction (RT-PCR). Diagnosis of rearrangements of *ETV6-RUNX1*, *BCR-ABL1*, and *MLL* were based on one or more of these techniques; diagnosis of HeH was defined by modal chromosomal number ≥52 or DNA index ≥1.13 for DS-ALL patients and ≥51 chromosomes for non-DS patients. All cytogenetic data were centrally reviewed by 2 coauthors (N.H. and E.F.). The definition and description of clonal abnormalities followed the recommendations of the International System for Human Cytogenetic Nomenclature (ISCN 2005).[39]

CRLF2 gene rearrangements were identified by genomic array, FISH, genomic PCR, Sanger sequencing, or multiplex ligation-dependent probe amplification.

Statistical analyses

Statistical analyses were conducted using SAS software (SAS-PC, version 9.1). The Kaplan-Meier method was used to estimate survival: complete remission rate (CR), event-free survival (EFS), overall survival (OS), and relapse-free survival (RFS). The survival estimates were compared using the log-rank test. The cumulative incidence of toxic death (TRM) and the cumulative incidence of relapse (CIR) were calculated by the method of

Kalbfleisch and Prentice and compared with the use of Gray's test. CR was defined as <5% blasts in the bone marrow, with regeneration of trilineage hematopoiesis plus absence of leukemic cells in the cerebrospinal fluid or elsewhere. EFS was calculated from the date of diagnosis to the date of last follow-up or to the first event, including relapse, death in CR, failure to achieve CR (considered as event on day 0), or second malignancy. Early death was defined as any death within the first 6 weeks of treatment and was considered as an event on day 0 for statistical analysis. OS was measured from the date of diagnosis to the date of last follow-up or to the date of death from any cause. CIR included death in CR and other events as competing events.

χ^2 analysis was used to compare categorical variables, and the Fisher exact test was used for small patient numbers. The nonparametric Mann-Whitney *U* test was applied for continuous variables. *P* values ≤ .05 were considered as statistically significant (2-tailed testing).

For multivariate analysis, the Cox regression model was used. Continuous variables were categorized according to the National Cancer Institute (NCI) risk criteria.[40] *P* values ≤ .05 were considered as statistically significant (2-tailed testing).

Results

Patient characteristics

In total, data of 708 DS-ALL patients were collected, of which 55 were excluded because they did not meet the inclusion criteria; ie, the karyotype of 1 patient lacked constitutional trisomy 21, 39 patients were diagnosed outside the inclusion period of the study, 2 patients were not treated with curative intent, and the age of 9 patients was <18 years at diagnosis (range, 18.2-21.9 years). Furthermore, we excluded the 5 patients with T-cell ALL because this number was considered too small for meaningful statistical analysis. However, clinical and cytogenetic characteristics of these 5 T-cell ALL patients are described in supplemental Table 4. Hence, 653 patients with DS BCP-ALL were analyzed. DS-ALL patients were slightly older than non-DS patients at diagnosis (median 5.0 vs 4.7 years; *P* = .002) (Table 1), and DS-ALL did not occur in infants. The initial white blood cell (WBC) count of DS-ALL patients was not different compared with non-DS (median 10.2 × 10^9/L [range 0.2-459] vs 8.9 × 10^9/L [range 1.7-998], *P* = .14).

Table 1. Patient characteristics of DS-ALL patients and the DCOG non-DS BCP-ALL reference cohort

	DS-ALL	non–DS-ALL	*P*
Number of patients	653	4445	—
Age at diagnosis (range), y	5.0 (1.2-17.9)	4.7 (0.1-17.9)	.002
Sex			
Male	343	2431	—
Female	310	2014	.3
Median initial WBC × 10^9/L (range)	10.5 (0.2-459)	8.8 (0.2-999)	.14
Extramedullary disease			
CNS (%)	16/624* (2.5)	98/4258* (2.2)	.69
Lymph nodes (%)	134/412* (32.5)	1471/4339* (33.1)	.57
Hepatomegaly (%)	245/469* (52.2)	3156/4357* (71)	<.001
Testis (%)	1/296* (<1%)	28/4317 (<1%)	.51
Cytogenetic subgroups			
Normal karyotype	179/444* (40.3)	45/650* (6.9)	<.001
BCR-ABL1 t(9;22)	3/444* (0.7)	93/3898* (2.4)	.02
MLL (11q23)	2/444* (0.5)	36/2966* (1.2)	.15
ETV6-RUNX1 t(12;21)	37/444* (8.3)	841/3264* (25.8)	<.001
HeH[$]	40/444* (9)	235/708* (33)	<.001
HeH trisomy 4 and 10	18 (4.5)	100 (42.5)	<.001
HeH, other	22 (5.5)	135 (57.5)	<.001
Others	183 (41.2)	225/650* (34.6)	.03
8-y OS	74% ± 2%	89% ± 2%	<.001
8-y EFS	64% ± 2%	81% ± 2%	<.001
8-y CIR	26% ± 2%	15% ± 1%	.001
2-y TRM	7% ± 1%	2% ± <1%	<.001

CNS, central nervous system involvement at diagnosis (>5 WBC/μL; CNS-3); HeH[$], DS: 52-60 chromosomes, non-DS: 51-60 chromosomes.
*Number of patients available for analysis.

Genetic data

All leukemic karyotypes and FISH and RT-PCR results underwent central review; 68% (n = 444) of the DS patients had adequate genetic data (Table 1). In total, 40.3% had a cytogenetically normal (CN) karyotype (ie, only constitutional trisomy 21) compared with 6.9% of the non-DS cases (*P* < .001). A total of 9% of DS patients had a HeH karyotype compared with 33% of non-DS patients (*P* < .001). HeH DS patients were significantly older than HeH non-DS patients (median 7.2 years vs 4.2, *P* < .001). Trisomies of both chromosomes 4 and 10 were found in 45% of the HeH DS-ALL patients, similar to non-DS HeH patients (42.6%, *P* = .77).[18,41]

ETV6-RUNX1 fusion was found in 8.3% of the DS-ALL patients (compared with 25.8% in non-DS, *P* < .001), *BCR-ABL1* fusion in 0.7% compared with 2.4% in non-DS (*P* = .02), and *MLL* rearrangements in <1% compared with 1.2% in non-DS (*P* = .2). The previously reported t(8;14)(q11.2;q32) translocation was found in DS-ALL patients only (2%).[2,42,43]

In total, 182 patients had available data on either *JAK* and/or *CRLF2* aberrations. *JAK2* R683 mutations were found in 21% (n = 30) of the 141 DS-ALL patients with available data, of which 83% (n = 25) also had a *CRLF2* gene rearrangement. In 69% (n = 93) of the 134 DS-ALL patients with available data, *CRLF2* gene rearrangements were found, including 5.4% (n = 6) with *IGH@-CRLF2* translocations and 94.6% (n = 87) with *P2RY8-CRLF2* fusions. DS patients with *CRLF2* gene rearrangements were younger compared with DS patients with wild-type *CRLF2* (4.1 vs 7.7 years, *P* < .001), but no difference in diagnostic WBC was observed (14.8 vs 11.8 × 10^9/L, *P* = .7). This differs from non-DS patients with *CRLF2* gene rearrangements who had lower WBC counts (14.6 vs 34.6 × 10^9/L, *P* = .004) but did not differ in age (5.1 vs 4.7 years, *P* = .7) compared with wild-type patients (supplemental Table 5).

Treatment outcome according to clinical data

The median follow-up time was 6.8 years for DS-ALL and 8.4 years for non-DS survivors. The CR rate was 96.7% in DS-ALL and 99% in non-DS patients (*P* < .001). Induction failures were more frequent in DS-ALL compared with non-DS (3.0% and 1.0%, respectively; *P* < .001). DS patients had a higher CIR (26% ± 2% vs 15% ± 1% at 8 years, *P* < .0001) and TRM (7% ± 1% vs 2% ± <1% at 2 years, *P* < .0001) than non-DS patients, resulting in a lower EFS (64% ± 2% vs 81% ± 2% at 8 years, *P* < .0001) and OS (74% ± 2% vs 89% ± 2%, *P* < .0001) (Figure 1). In total, 144 DS patients relapsed compared with 650 non-DS patients. The time to relapse after CR was significantly longer for DS (median 2.8 years, p25: 1.8 years, p75: 4.0 years) than for non-DS patients (median 2.4 years, p25: 1.4 years, p75: 3.5 years, *P* = .007). In addition, 23 DS-ALL patients relapsed after 5 years vs 33 non–DS-ALL patients (*P* < .001). Treatment outcome did not differ significantly between the early (1995-2000) and late (2000-2004) treatment eras for DS patients (8-year: OS 77% ± 3% vs 73% ± 3%, *P* = .7; CIR 26.7% ± 3% vs 31% ± 6%, *P* = .4).

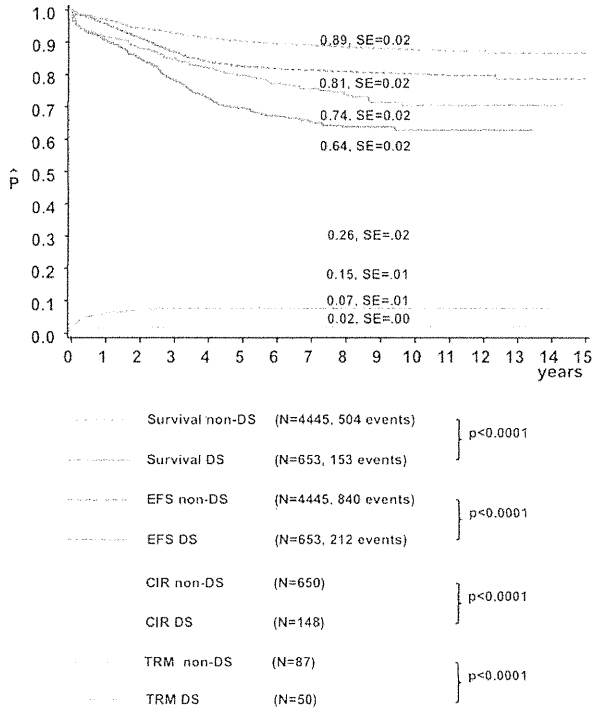

Figure 1. Treatment outcome of the DS-ALL and non–DS-ALL patients. The continuous lines represent DS-ALL patients, and the dotted lines represent non–DS-ALL patients. The red line represents OS, the blue line EFS, the green line the cumulative incidence of TRM, and the light blue line CIR. SE, standard error.

The 379 DS-ALL children <6 years fared significantly better than the 272 older children (8-year: EFS 70% ± 3% vs 54% ± 4%, $P < .0001$; OS 78% ± 2% vs 67% ± 3%, $P = .002$; CIR 21% ± 2% vs 34% ± 3%, $P \leq .001$; and 2-year cumulative incidence of TRM, 7% ± 1% vs 8% ± 2%; $P = .33$). Notably, the 126 children aged 6 to 9 years had a relatively poor outcome (8-year: EFS 51% ± 3%, OS 70% ± 5%), which was due to a very high frequency of relapse (CIR 41% ± 6%) not attributable to any known risk factor(s). Outcome declined with increasing WBC count and was best for the 319 patients with WBC $<10 \times 10^9$/L due to a low risk of TRM (8-year: 4% ± 1% vs 11% ± 2% for WBC $\geq 10 \times 10^9$/L, $P = .0003$) and relapse (8-year: 21% ± 3% vs 30% ± 3%, $P = .03$). These features thus define a favorable risk group with age <6 years and WBC $<10 \times 10^9$/L, when compared with the remaining DS patients (8-year: EFS 78% ± 3% vs 58% ± 3%, $P < .0001$; OS 87% ± 3% vs 68% ± 3%, $P < .0001$; CIR 17% ± 3% vs 30% ± 2%, $P = .003$; 2-year TRM 3% ± 1% vs 9% ± 1%, $P = .002$) (Figure 2, Table 2). These criteria predicted outcome more accurately than the classical NCI criteria (supplemental Figure 1).[40] These features remained significant after excluding patients with *ETV6-RUNX1* rearrangements or trisomies 4 and 10 from the analysis. The effect of this new Ponte di Legno (PdL) risk stratification was consistent among the larger study groups including AIEOP, BFM, CCG, POG, and the UK with a hazard ratio (HR) of 1.62 for high-risk patients from the UK and 3.79 for BFM patients. Among patients with age >6 years and WBC $>10 \times 10^9$/L, DS patients had a poorer outcome than non-DS patients (8-year: EFS 58% ± 3% vs 78% ± 1%, $P < .001$; OS 68% ± 3% vs 86% ± 1%, $P < .001$; CIR 30% ± 2% vs 17% ± 1%, $P < .001$; 2-year TRM 10% ± 1% vs 2% ± <1%, $P < .0001$). The clinical characteristics of DS-ALL patients (n = 246) classified as NCI low risk but considered high risk according to our criteria are described in supplemental Table 6.

In total, 18 (2.8%) of the DS-ALL patients received a stem cell transplantation: 3 in CR1 and 15 in CR2. Of these patients, 6 are alive in continuous CR and 12 patients died (1 graft-versus-host disease, 1 toxic noninfectious event, 1 infection, and 9 relapses).

Treatment outcome according to genetic data

The 37 DS-ALL patients with *ETV6-RUNX1* had significantly better outcome than the other DS patients: 8-year EFS 95% ± 4% vs 63% ± 3% ($P = .001$), OS 97% ± 3% vs 75% ± 2% ($P = .007$), CIR 3% ± 3% vs 26% ± 2% ($P = .004$), and 2-year TRM 3% ± 3% vs 8% ± 1%; ($P = .2$). DS-ALL patients with *ETV6-RUNX1* did not differ in outcome when compared with the 841 non-DS patients with this abnormality (8-year: EFS 95%, $P = .48$; OS 96%, $P = .91$; CIR 7%, $P = .32$; 2-year TRM 1%, $P = .19$).

The 40 HeH DS-ALL patients had a significantly lower CIR than the other DS-ALL patients (8-year: 8% ± 5% vs 26% ± 3%, $P = .02$). However, a relatively high rate of TRM (2-year: 13% ± 5% in HeH vs 7% ± 1% in non-HeH DS, $P = .2$) resulted in similar 8-year EFS (77% ± 7% vs 65% ± 3%, $P = .28$) and OS (79% ± 6% vs 76% ± 2%, $P = .88$). TRM in these HeH patients was not exclusively seen in one treatment strategy but was spread across the different treatment protocols. HeH DS-ALL patients showed lower OS when compared with the 235 HeH non-DS patients due to increased TRM (8-year: OS 79% ± 6% vs 93% ± 2%, $P = .009$; EFS 77% ± 7% vs 86% ± 2%, $P = .06$; CIR 8% ± 5% vs 11% ± 2%, $P = .7$; 2-year: TRM 13% ± 5% vs 1% ± 1%, $P < .001$).

The subgroup of HeH DS-ALL patients with trisomies 4 and 10 (n = 18) showed a trend toward better outcome when compared with all other DS-ALL patients (8-year: EFS 88% ± 8% vs 65% ± 3%, $P = .09$; OS 88% ± 8% vs 76% ± 2%, $P = .32$; CIR 0% vs 25% ± 2%, $P = .03$; 2-year: TRM 12% ± 8% vs 7% ± 1%, $P = .6$). No DS patients with these trisomies relapsed, and all events were due to toxicity. Their outcome was similar when compared with non-DS patients with trisomy 4 and 10 (8-year: EFS 90.8% ± 3%; $P = .75$, OS 92.3% ± 4%; $P = .65$, CIR 5.1% ± 2%; $P = .34$, 2-year: TRM 3.0% ± 2%, $P = .1$).

DS-ALL patients with or without *JAK2* mutations had similar treatment 8-year outcomes (EFS 57% ± 10% vs 69% ± 5%, $P = .1$; CIR 26% ± 9% vs 23% ± 5%, $P = .48$). No data were available in the reference cohort. The 93 DS-ALL patients with *CRLF2* aberrations showed no significant difference in 8-year survival compared with the 41 wild-type DS-ALL patients (EFS 62% ± 6% vs 71% ± 8%, $P = .21$; OS 73% ± 5% vs 83% ± 8%, $P = .13$; CIR 26% ± 6% vs 22% ± 8%, $P = .44$). DS-ALL patients with *CRLF2* gene rearrangements did not differ in outcome from non–DS-ALL patients with these aberrations (8-year: EFS 62% ± 6% vs 58% ± 9%, $P = .7$; OS 73% ± 5% vs 79% ± 8%, $P = .6$; CIR 26% ± 6% vs 38% ± 9%, $P = .15$). Median time to relapse for DS patients with *CRLF2* aberrations was 29 months vs 51 months in patients with wild-type *CRLF2* ($P = .11$).

Treatment-related mortality

In total, 7.7% of the DS-ALL patients died of other causes than relapsed/refractory disease compared with 2.3% in non-DS ($P < .001$). TRM occurred at all phases of therapy, including maintenance (supplemental Table 7). TRM death during induction occurred in 2.8% (n = 18) of the DS patients (13 infectious, 5 noninfectious deaths). In CR, 4.9% (n = 32) of the DS patients died of TRM (25 infectious, 7 noninfectious). The most common cause of TRM

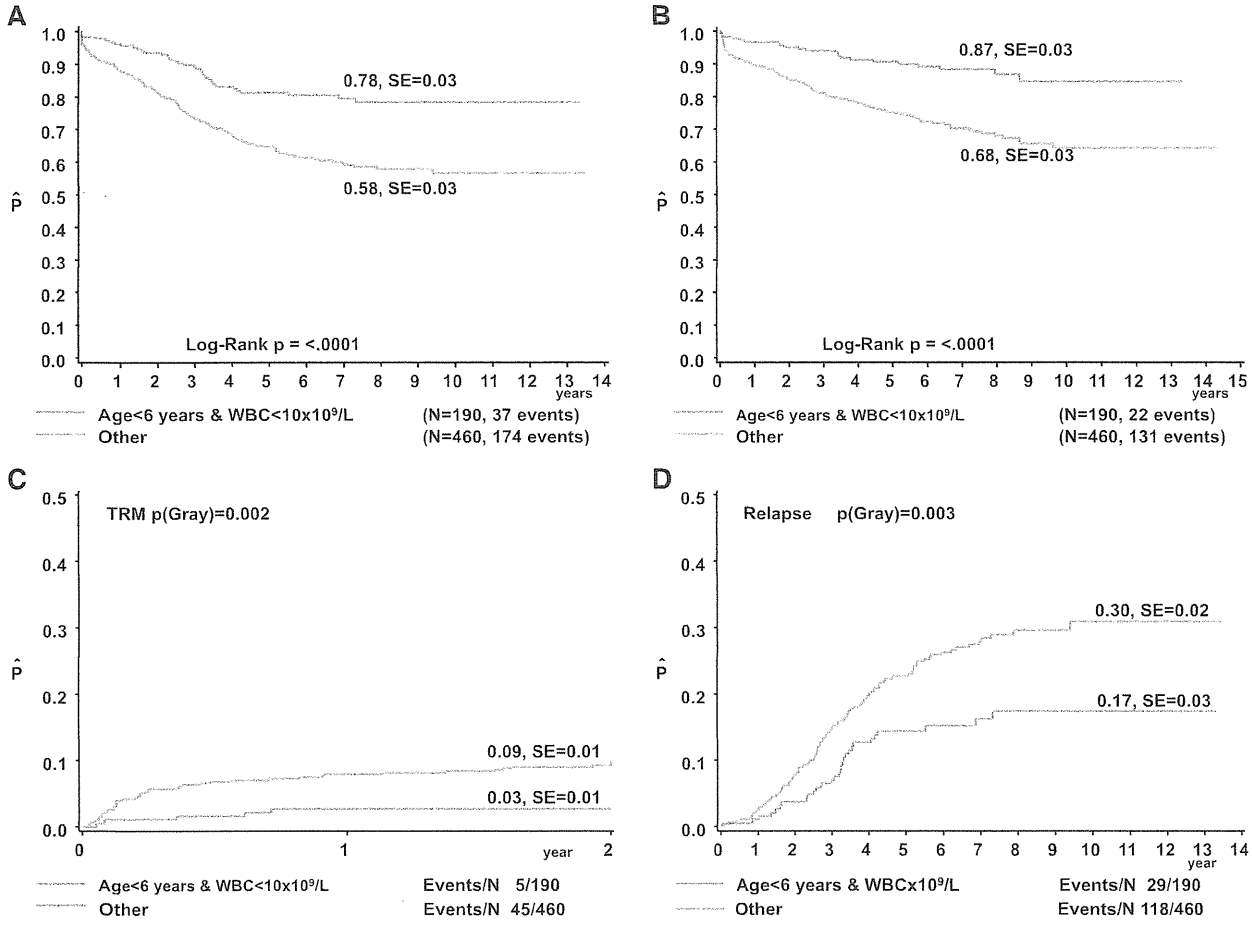

Figure 2. Treatment outcome according to age and WBC in DS-ALL. The OS (A), EFS (B), cumulative incidence of TRM (C), and CIR (D) are depicted for patients aged <6 years with WBC $<10 \times 10^9$/L (blue line) vs all other DS-ALL patients (red line). The numbers on the curves for OS and EFS represent results at 8 years. The numbers on the curves for TRM are 2-year results (during treatment only) and those for relapse are results at 8 years. SE, standard error.

was infection, mainly respiratory and bacterial infections. Only 0.3% (n = 2) of the DS ALL patients died of second malignancies in CR1 (secondary AML and Epstein-Barr virus lymphoproliferative disease), compared with 1.3% of the non-DS patients ($P < .04$). Secondary malignancies in non-DS patients included 28 AML/myelodysplastic syndromes, 5 brain tumors, 9 other tumors, and 13 other malignancies.

Table 2. Contingency table representing outcome of DS patients by NCI risk group and PdL risk group criteria

	PdL risk criteria		
Classical NCI criteria	Standard risk	High risk	
Low risk	n = 187	n = 246	NCI SR
	EFS 78% ± 3%	EFS 63% ± 4%	n = 433
	OS 87% ± 3%	OS 73% ± 3%	
	TRM 3% ± 1%	TRM 7% ± 2%	
	CIR 17% ± 3%	CIR 30% ± 4%	
High risk	n = 0	n = 218	NCI HR
		EFS 57% ± 4%	n = 218
		OS 62% ± 4%	
		TRM 12% ± 2%	
		CIR 29% ± 3%	
	PdL SR model	PdL HR model	Total
	n = 187	n = 464	n = 651

Classical NCI risk criteria: age 1-9 or ≥ 10 y at diagnosis and WBC < or ≥50 × 10^9/L; PdL low-risk criteria: age <6 y and WBC $<10 \times 10^9$/L; PdL high-risk criteria: all other patients.

HR, high risk; SR, standard risk.

TRM was not significantly different between DS patients treated in the CCG/POG/UK studies (3-drug induction) and those DS patients treated in AIEOP/BFM studies (4-drug induction); the rate of death during induction was 1.1% ± 1% vs 1.9% ± 1% ($P = .7$) and the 2-year cumulative rate was 7% ± 2% vs 8% ± 3% ($P = .99$). The inclusion of an anthracycline in induction (4-drug induction) had no impact on TRM.

Multivariate analysis

Stepwise multivariate Cox regression analysis of EFS revealed age <6 years (HR = 0.58, 95% confidence interval [CI] = 0.41-0.81, $P = .002$), WBC $<10 \times 10^9$/L (HR = 0.60, 95% CI = 0.42-0.86, $P = .005$), and *ETV6-RUNX1* (HR = 0.14, 95% CI = 0.03-0.57, $P = .006$) as independent predictors for favorable outcome. They also independently predicted OS (age HR = 0.66, $P = .04$; WBC $<10 \times 10^9$/L HR = 0.51, $P = .003$; and *ETV6-RUNX1* HR = 0.12, $P = .04$). RFS was predicted by age, *ETV6-RUNX1*, and HeH (Table 3).

In non–DS-ALL, the classical NCI criteria are age and the initial WBC count; however, *ETV6-RUNX1* and trisomy 4 and 10 are independent predictors for favorable outcome (*ETV6-RUNX1*: HR = 0.29, 95% CI = 0.15-0.58, $P < .001$; or trisomy 4 and 10: HR = 0.37, 95% CI = 0.17-0.79, $P = .011$). NCI criteria retained

Table 3. Multivariate analysis of the DS-ALL data set

Outcome	Variable	HR	95% CI	*P* value
	Age <6 y	0.58	0.41-0.81	.002
EFS	WBC <10 × 10^9/L	0.60	0.42-0.86	.005
	ETV6-RUNX1	0.14	0.03-0.57	.006
	HeH	0.68	0.34-1.36	.275
	Age <6 y	0.66	0.44-0.99	.044
OS	WBC <10 × 10^9/L	0.51	0.33-0.79	.003
	ETV6-RUNX1	0.12	0.02-0.86	.035
	HeH	1.01	0.48-2.11	.983
	Age <6 y	0.48	0.32-0.73	.000
RFS	WBC <10 × 10^9/L	0.71	0.46-1.08	.105
	ETV6-RUNX1	0.10	0.01-0.64	.016
	HeH	0.29	0.09-0.92	.036

HeH ≥ 52 chromosomes.

their prognostic value in a Cox model with these 3 variables (HR = 1.96; 95% CI = 1.30-2.95, P = .001). In addition, multivariate analysis showed that the PdL criteria are not driven by the large group of DS-ALL patients having *CRLF2* aberrations (HR = 0.66, 95% CI = 0.33-1.33, P = .25), but more likely by age and initial WBC (HR = 2.16, 95% CI = 0.95-4.90, P = .07).

Discussion

Many study groups have reported a worse clinical outcome for DS-ALL, but almost all reports lack sufficient power to answer relevant biological questions in DS-ALL, which is the reason the PdL group undertook this retrospective review. The unprecedented size of this study cohort resolves the controversy of the frequency and clinical impact of specific (cyto)genetic aberrations in DS-ALL.[2,18] Moreover, the scale of the study enabled the identification of relatively small subgroups of DS-ALL with favorable outcomes. Analysis of 444 DS-ALL patients with known cytogenetics demonstrated that the genetic subgroups predicting favorable outcome in non–DS-ALL[2,6,7,18,41,44] also predict favorable outcome in DS-ALL. Most significant is the discovery that *ETV6-RUNX1* conferred an excellent prognosis and that HeH with trisomy of chromosomes 4 and 10 was associated with a very low CIR. Hence, these patients, comprising 12% of DS-ALL, may be eligible for future treatment reduction to reduce TRM and can be treated according to the same risk-stratified algorithms as non-DS patients in the collaborative study group protocols.

Another novel finding of this study was the identification of a clinically favorable prognostic subgroup of DS-ALL patients, characterized by age <6 years and WBC <10 × 10^9/L. These cut points differ from those used in the classical NCI ALL risk criteria, although the biological basis for this difference is not fully understood.[40] No genetic abnormalities were identified that could explain this difference between the classical NCI and the herein-reported criteria. Remarkably, children aged between 6 and 9 years at diagnosis had a relatively poor outcome similar to high-risk ALL patients, which was due to a high frequency of relapse. This subgroup may be treated according to a medium- or high-risk arm of future collaborative study group protocols. Unraveling the genetic background of the leukemia in this subgroup will be required in order to design more rational therapy for these patients. Noteworthy, minimal residual disease (MRD) was not routinely determined during the era of this study, and it is unclear whether MRD would confirm these novel risk groups. Because MRD was proven to be a powerful tool in non–DS-ALL risk assignment,[45,46] further research is needed to validate whether an MRD-based strategy is desirable in future DS-ALL treatment protocols.

In general, we showed that DS-ALL patients have an inferior survival when compared with a representative non–DS-ALL cohort treated in the same time period, which is in agreement with previous smaller studies.[5,10,47] Despite a high rate of TRM, and different from what is often suggested, relapse remained the main cause of treatment failure in DS patients. Interestingly, the relapses tend to occur later in DS. It is unclear if this is due to the genetic makeup of DS-ALL or to decreased immune surveillance of the residual leukemia in DS patients. It cannot be ruled out that underreported treatment reduction of patients with DS-ALL contributes to the increased relapse risk.[48] This finding suggests that the currently accepted strategy of treatment reduction in DS-AML, which is characterized by a chemotherapy-sensitive phenotype,[49] is not applicable to DS-ALL.[47] The only exception may be DS-ALL patients with *ETV6-RUNX1* or HeH, in which TRM outweighed the risk of relapse, for whom a 3-drug induction and a limited reinduction might be adequate. Interestingly, and in accordance with previous results, the incidence of secondary malignancies was significantly lower in DS patients as compared with non–DS-ALL patients. This is in agreement with the reduced propensity for solid tumors in DS patients reported before.[50]

The genetic basis of the aggressive clinical behavior of DS-ALL is still unknown. A high proportion of DS-ALLs have normal karyotype (40.3% compared with 6.9% of non-DS), suggesting the presence of cytogenetically invisible molecular abnormalities. One of these abnormalities, detected in 60% of DS-ALLs, is the aberrant expression of *CRLF2*, which is often associated with *JAK-STAT* mutations. In contrast to some studies showing deleterious effects of *CRLF2* alterations in non-DS high-risk ALL,[26,51] no such association was found in this study or in several prior smaller studies of DS-ALL.[3,4,21,27] Nevertheless, a substantial proportion of DS-ALL patients carry these aberrations, thereby providing a pathway that might be targeted by inhibitors of the JAK-STAT pathway or mTOR signaling.[52]

IKZF1 mutational status was unknown in our data set. Recently, it was shown that this gene was frequently deleted in DS-ALL patients (in ~35%) and was found to be an independent predictor for dismal outcome.[27] Of note, the median age of patients with *IKZF1* aberrations in the DS-ALL study was significantly higher compared with wild-type patients (8.2 vs 4.3 years), which could be an important genetic factor underlying the biological basis for the age cutoff point of 6 years reported here as clinically significant.

Previous studies reported increased TRM in children with DS-ALL,[9] also in relapse protocols.[53] The large size of our cohort enabled the observation that the increased TRM is present throughout treatment, with about half of the deaths occurring during maintenance therapy. While doses of myelosuppressive chemotherapy are typically adjusted during maintenance therapy, to maintain an adequate neutrophil count, this phase of treatment may nevertheless lead to B-cell depletion and hypogammaglobulinemia, and hence to a higher infection rate in already immune-compromised DS patients.[54,55] To reduce TRM, we suggest improving supportive care throughout the treatment period with aggressive treatment of infections and studies analyzing the potential benefit of antibacterial and antifungal prophylaxis and/or immunoglobulin substitution. Patients should be leukocyte depleted as non-DS patients during maintenance in order to prevent relapse, but with prompt interruptions for aplasia and with more intensive surveillance than non-DS children.

In conclusion, this large international study demonstrated that the poorer survival seen in DS-ALL is mainly due to a higher relapse rate and less so to TRM. Therefore, treatment reduction is not warranted, except for the 12% of patients with HeH or *ETV6-RUNX1* in which toxicity is the major cause of mortality. Because TRM occurs throughout therapy and is not associated with a specific chemotherapy regimen, better surveillance and improved supportive care measures throughout therapy need to be evaluated. As a result of this study, an initiative is underway to develop an international treatment protocol for children with DS-ALL.

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Authorship

Contribution: T.D.B., S.I., J.W., C.H.P., and C.M.Z. conceived of, designed, and wrote the manuscript; T.D.B., S.I., M.Z., E.F., N.A.H., M.M.v.d.H.-E., R.P., C.M.K., L.B.S., K.S., D.-C.L., K.H., M.A., A.B., G.B., K.R.R., M.S., G.C., G.M., M.M., R.P.-G., V.M., T.L., H.C., B.S., I.G., A.V.M., A.V., S.P.H., C.-H.P., C.G.M., A.M., G.E., J.R.K., J.A.W., and C.M.Z. provided all study materials or patients; T.D.B., S.I., J.W., and C.M.Z. collected and assembled data; T.D.B., S.I., M.Z., J.W., N.H., E.F., M.M.v.d.H.-E., and C.M.Z. analyzed and interpreted data; and all authors gave final approval of the manuscript.

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Correspondence: C. M. Zwaan, Erasmus MC–Sophia Children's Hospital, Department of Pediatric Oncology and Haematology, Dr. Molewaterplein 60, 3015 GJ Rotterdam, The Netherlands, e-mail: c.m.zwaan@erasmusmc.nl; and Shai Izraeli, Functional Genomics and Childhood Leukemia Research, Sheba Medical Center Tel-Hashomer, Ramat Gan, Israel 52621; e-mail: shai.izraeli@sheba.health.gov.il.

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