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


1Pediatric Oncology/Hematology, Erasmus MC–Sophia Children’s Hospital, Rotterdam, the Netherlands.
2Department of Paediatric Haematology-Oncology, Edmond and Lily Safra Children’s Hospital, Sheba Medical Centre, Tel-Ha‘asomer, Ramat Gan, and Tel Aviv University Medical School, Tel Aviv, Israel.
3Statistical Office of Berlin-Frankfurt-Münster (BFM) Study Group, Pediatric-Hematology/Oncology, Medical School Hannover, Hannover, Germany.
4Department of Medical Biosciences, University of Umeå, Umeå, Sweden.
5Department of Pathology, The Ohio State University, Columbus, United States.
6Dutch Childhood Oncology Group, The Hague, the Netherlands.
7Dana–Farber Cancer Institute, Boston, MA, United States.
8Pediatric and Adolescent Medicine, the Juliane Marie Centre, the University Hospital Rigshospitalet, Copenhagen, Denmark.
9Pediatric Hematology-Oncology Division, Mackay Memorial Hospital, Taipei, Taiwan.
10Clinical Research Center, National Hospital Organization, Nagoya Medical Center, Nagoya, Aichi, Japan.
11Department Pediatric Hematology Oncology, Azienda Ospedaliero-Universitaria Meyer Children Hospital, Florence, Italy.
12Department of Pediatrics, Uniβersity of Milano-Bicocca, Ospedale S. Gerardo, Monza, Italy.
13Pediatric Hemato-Oncology, Department of Pediatrics “Salus Pueri”, University of Padua, Padova, Italy.
14Division of Pediatric Hematology/Oncology, Texas Children’s Cancer Center, Baylor College of Medicine, Houston, Texas, United States.
15Department of Pediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Germany.
16Children’s Cancer Research Institute, St Anna Children’s hospital, university medical school Vienna, Austria.
17Department of Pediatric Hemato-Oncology, Ghent University Hospital, Belgium.
19Center of Pediatric Hematology/Oncology, Schneider Children’s Medical Center of Israel, Petah Tiqva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.
20Leukaemia Research Cytogenetics Group, Northern Institute for Cancer Research, Newcastle University, Newcastle-upon-Tyne, United Kingdom.
21Department of Haematology, Sheffield Children’s Hospital, Sheffield, United Kingdom.
22Department of Pediatrics, University of Colorado School of Medicine and Children’s Hospital, Aurora, Colorado, United States.
23Department of Oncology and Pathology, St. Jude Children’s Research Hospital, Memphis, Tennessee, United States.
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Please address all correspondence to:
C.M. Zwaan, MD, PhD
Erasmus MC - Sophia Children's Hospital
Dept. of Pediatric Oncology and Haematology
Dr. Molewaterplein 60
3015 GJ Rotterdam
The Netherlands
Phone: +31-10-703.6691
Fax: +31-10-703.1134
E-mail: c.m.zwaan@erasmusmc.nl

Shai Izraeli, MD
Head, Functional Genomics and childhood leukemia research
Sheba Medical Center Tel-Hashomer, Ramat Gan, Israel 52621
Professor of Pediatrics
Dpt. Human Molecular Genetics and Biochemistry Tel Aviv University
Tel 972-3-5303037/972-52-666360
Email: Shai.Izraeli@sheba.health.gov.il
Key points

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

Children with Down syndrome (DS) have an increased risk of B-cell precursor acute lymphoblastic leukemia (BCP-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 1995-2004. Non-DS BCP-ALL patients from the DCOG and BFM were reference cohorts. DS-ALL patients had a higher 8-year cumulative incidence of relapse (26±2% vs. 15±1%; p<0.001) and 2-year treatment-related mortality (TRM) (7±1% vs. 2.0±1%; p<0.0001) than non-DS patients, resulting in lower 8-year event-free survival (EFS) (64±2% vs. 81±2%; p<0.0001) and overall survival (74±2% vs. 89±1%; p<0.0001). Independent favorable prognostic factors include age<6 years (hazard ratio [HR]=0.58, p=0.002), white blood cell count (WBC) <10x10^9/L (HR=0.60, p=0.005) and ETV6-RUNX1 (HR=0.14; p=0.006) for EFS, age (HR=0.48, p<0.001), ETV6-RUNX1 (HR 0.1, p=0.016) and high hyperdiploidy (HeH) (HR 0.29, p=0.04) 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.
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 to non-DS patients because of both higher treatment related mortality (TRM) and higher relapse rate.\(^5\)-\(^9\) As 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.\(^5\)-\(^10\) Small series suggest that DS-ALL patients have an increased risk of mucositis from methotrexate, 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, as 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 the t(12;21)(p13;q22) [ETV6-RUNX1], high hyperdiploidy (HeH) and trisomies 4&10 is uncertain in DS-ALL, as well as for the unfavorable translocations including the 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 mentioned in Supplementary table 1. A predefined set of data was collected, consisting of clinical data obtained at diagnosis, treatment, and cytogenetic and molecular data (Supplementary 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 10-75% of the maximum dose, and intensified leucovorin rescue. DS ALL patients enrolled in EORTC 58951 protocols from September 2002 (n=7) received 0.5g/m² of MTX instead of 5g/m². In addition, patients treated on protocol POG 9405 (n=10) started with 50% of the total dose of Daunorubicin, Cytarabine, Teniposide, HDAC and Peg-asparaginase, which was successively increased or reduced depending on toxicity. Supplementary 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. However, several study groups contributed new unpublished data.
Non-DS ALL reference cohort

For comparison, population-based B-cell precursor ALL reference cohorts from the DCOG and the ALL-BFM Study Group, from exactly the same time period as the DS patients (January 1, 1995 and December 31, 2004), were added. The DCOG dataset consisted of 827 non-DS BCP-ALL patients enrolled in 3 DCOG ALL treatment protocols (ALL8, ALL9 and ALL10). The BFM dataset 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 on-going.29,30

There were no significant differences in outcome estimates, nor in the distribution of cytogenetic subgroups, between the DCOG and BFM datasets (data not shown), nor when compared to reported data from other participating groups.31-38 The DCOG and BFM non-DS datasets 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-transcribed 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 high hyperdiploidy (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 two co-authors (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).19

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), 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 less than 5% blasts in the bone marrow, with regeneration of tri-lineage 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.
To compare categorical variables $\chi^2$ analyses was used and the Fisher exact test was used for small patient numbers. The non-parametric Mann-Whitney U test was applied for continuous variables. $P$ values $\leq 0.05$ were considered as statistically significant (two-tailed testing).

For multivariate analysis, the Cox regression model was used. Continuous variables were categorized according to the National Cancer Institute (NCI) risk criteria. $P$ values $\leq 0.05$ were considered as statistically significant (two-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; i.e. the karyotype of one 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 above 18 years at diagnosis (range 18.2 – 21.9). Furthermore, we excluded the 5 patients with T-cell ALL as this number was considered too small for meaningful statistical analysis. However, clinical and cyto-genetic characteristics of these 5 T-cell ALL patients are described in Supplementary 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=0.002) (Table 1), and DS-ALL did not occur in infants. The initial white blood-cell count (WBC) of DS-ALL patients was not different compared with non-DS (median 10.2x10^9/L (range 0.2–459) vs. 8.9x10^9/L (range 1.7–998), p=0.14).

Genetic data

All leukemic karyotypes, 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 (i.e. only constitutional trisomy 21) compared to 6.9% of the non-DS cases (p<0.001). Nine percent of DS patients had a HeH karyotype compared to 33% of non-DS patients (p<0.001). HeH DS patients were significantly older than HeH non-DS patients (median, 7.2 years vs. 4.2; p<0.001). 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=0.77). 10,41

ETV6-RUNX1 fusion was found in 8.3% of the DS-ALL patients (compared with 25.8% in non-DS; p<0.001), BCR-ABL1 fusion in 0.7% compared with 2.4% in non-DS (p=0.02) and MLL rearrangements in <1% compared with 1.2% in non-DS (p=0.2). 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 to DS patients with wildtype CRF2 (4.1 vs. 7.7 years, p<0.001), but no difference in diagnostic WBC was observed (14.8 vs. 11.8x10^9/L, p=0.7). This differs from non-DS patients with CRLF2 gene rearrangements who had lower WBC (14.6 vs. 34.6x10^9/L, p=0.004), but did not differ in age (5.1 vs. 4.7 years, p=0.7) compared to wild-type patients (Supplementary 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<0.001). Induction failures were more frequent in DS-ALL compared to non-DS (3.0% and 1.0% respectively, p<0.001). DS patients had a higher cumulative incidence of relapse (CIR, 26±2% vs. 15±1% at 8 years; p<0.0001), and treatment related mortality (TRM, 7±1% vs. 2±1% at 2 years; p<0.0001) than non-DS patients, resulting in a lower EFS (64±2% vs. 81±2% at 8 years; p<0.0001) and OS (74±2% vs. 89±2%; p<0.0001) (Figure 1). In total, 144 DS patients relapsed compared to 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=0.007). In addition, 23 DS ALL patients relapsed after 5 years versus 33 non-DS ALL patients, p<0.001. Treatment outcome did not differ significantly between the early (1995-2000) and late treatment eras (2000-2004) for DS patients (8-year: OS 77±3% vs. 73±3%; p=0.7, CIR 26.7±3% vs. 31±6%; p=0.4).

The 379 DS-ALL children below the age of 6 years fared significantly better than the 272 older children (8-year: EFS 70±3% vs. 54±4%; p<0.0001; OS 78±2% vs. 67±3%; p=0.002, CIR 21±2% vs. 34±3%; p<0.001, and 2-year cumulative incidence of TRM 7±1% vs. 8±2%; p=0.33). Notably, the 126
children aged 6-9 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, and was best for the 319 patients with WBC <10x10^9/L due to a low risk of TRM (8-year: 4±1% vs. 11±2% for WBC ≥10x10^9/L; p=0.0003) and relapse (8-year: 21±3% vs. 30±3%; p=0.03). These features thus define a favorable risk-group with age <6 years and WBC <10x10^9/L, when compared to the remaining DS patients (8-year: EFS: 78±3% vs. 58±3%, p<0.0001; OS: 87±3% vs. 68±3%, p<0.0001; CIR: 17±3% vs. 30±2%, p=0.003; 2-year TRM: 3±1% vs. 9±1%, p=0.002) (Figure 2, Table 2). These criteria predicted outcome more accurately than the classical NCI-criteria (Supplementary Figure 1). These features remained significant after excluding patients with ETV6-RUNX1 rearrangements or trisomies 4&10 from the analysis. The effect of this new PdL risk stratification was consistent among the larger study groups including AIEOP, BFM, CCG, POG, and the UK with a 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 >10x10^9/L, DS patients had a poorer outcome than non-DS patients (8-year: EFS: 58±3% vs. 78±1%, p<0.001; OS: 68±3% vs. 86±1%, p<0.001; CIR: 30±2% vs. 17±1%, p<0.001; 2-year TRM: 10±1 vs. 2±<1%, p<0.0001). 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 Supplementary 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 non-infectious event, 1 infection, and 9 relapsed).

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=0.001), OS 97±3% vs. 75±2% (p=0.007), CIR 3±3% vs. 26±2% (p=0.004), and 2-year: TRM 3±3% vs. 8±1%; (p=0.2). DS ALL patients with ETV6-RUNX1 did not differ in outcome when compared to the 841 non-DS patients with this abnormality (8-year: EFS 95%,
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=0.02). However a relatively high rate of TRM (2-year: 13±5% in HeH vs. 7±1% in non-HeH DS; p=0.2) resulted in similar 8-year EFS (77±7% vs. 65±3%, p=0.28) and OS (79±6% vs. 76±2%, p=0.88). 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 to the 235 HeH non-DS patients due to increased TRM (8-year: OS 79±6% vs. 93±2%; p=0.009, EFS 77±7% vs. 86±2%; p=0.06, CIR 8±5% vs. 11±2%; p=0.7, 2-year: TRM 13±5% vs. 1±1%, p<0.001).

The subgroup of HeH DS-ALL patients with trisomies 4&10 (n=18) showed a trend towards better outcome, when compared to all other DS-ALL patients (8-year: EFS 88±8% vs. 65±3%, p=0.09; OS 88±8% vs. 76±2%, p=0.32; CIR 0% vs.25±2%, p=0.03; 2-year: TRM 12±8% vs.7±1%, p=0.6). No DS patients with these trisomies did relapse, and all events were due to toxicity. Their outcome was similar when compared to non-DS patients with trisomy 4&10 (8-year: EFS 90.8±3%; p=0.75, OS 92.3±4%; p=0.65, CIR 5.1±2%; p=0.34, 2-year: TRM 3.0±2%, p=0.1).

DS ALL patients with or without JAK2 mutations had similar treatment 8-year outcomes (EFS 57±10% vs. 69±5%, p=0.1; CIR 26±9% vs. 23±5%, p=0.48). 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 to the 41 wild-type DS ALL patients (EFS 62±6% vs. 71±8%, p=0.21; OS 73±5% vs. 83±8%, p=0.13; CIR 26±6% vs. 22±8%; p=0.44). 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=0.7; OS 73±5% vs. 79±8%; p=0.6; CIR 26±6% vs. 38±9%; p=0.15). Median time to relapse for DS patients with CRLF2 aberrations was 29 months versus 51 months in patients with wildtype CRLF2 (p=0.11).
Treatment related mortality

In total, 7.7% of the DS-ALL patients died from other causes than relapsed/refractory disease compared to 2.3% in non-DS (p<0.001). TRM occurred at all phases of therapy, including maintenance (supplementary Table 7). TRM death during induction occurred in 2.8% (n=18) of the DS patients (13 infectious, 5 non-infectious deaths). In CR, 4.9% (n=32) of the DS patients died of TRM (25 infectious, 7 non-infectious). The most common cause of TRM 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 to 1.3% of the non-DS patients, p<0.04. Secondary malignancies in non-DS patients included 28 AML/MDS, 5 brain tumors, 9 other tumors, and 13 other malignancies.

TRM was not significantly different between DS patients treated on the CCG/POG/UK studies (3-drug induction) and those DS patients treated on AIEOP/BFM-studies (4-drug induction): the rate of death during induction was 1.1±1% vs. 1.9±1% (p=0.7) and the 2-year cumulative rate was 7±2% vs. 8±3 % (p=0.99). 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%CI= (0.41–0.81); p=0.002), WBC <10x10^9/L (HR=0.60; 95%CI= (0.42–0.86); p=0.005), and ETV6-RUNX1 (HR=0.14; 95%CI=(0.03–0.57); p=0.006) as independent predictors for favorable outcome. They also independently predicted OS (Age HR=0.66, p=0.04; WBC<10x10^9/L HR=0.51 p=0.003; and ETV6-RUNX1 HR=0.12, p=0.04). Relapse-free survival (RFS) was predicted by age, ETV6-RUNX1, and HeH (Table 3).

In non-DS ALL the classical NCI criteria are comprised by age and the initial WBC, however ETV6-RUNX1 and trisomy 4&10 are independent predictors for favorable outcome (ETV6-RUNX1: HR=0.29; 95%CI= (0.15–0.58); p<0.001; or trisomy 4&10: HR=0.37; 95%CI= (0.17–0.79); p=0.011).
NCI-criteria retained their prognostic value in a Cox model with these three variables (HR 1.96; 95%CI= (1.30-2.95), p=0.001). In addition, multivariate analysis showed that the PdL criteria are not driven by the large group of DS-ALL patients having \textit{CRLF2} aberrations (HR=0.66; 95%CI= (0.33–1.33); p=0.25), but more likely by age and initial WBC (HR=2.16; 95%CI= (0.95–4.90); p=0.07).
Discussion

Many study groups have reported the worse clinical outcome of DS-ALL, however, almost all reports lack sufficient power to answer relevant biological questions in DS-ALL, which is the reason the Ponte di Legno 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. 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, 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&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 <10x10^9/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. 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, MRD was not routinely determined during the era of this study, and it is unclear whether MRD would confirm these novel risk-groups. Since MRD was proven to be a powerful tool in non-DS ALL risk assignment, further research is needed to validate whether a 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 to a representative non-DS ALL cohort treated in the same time period, which is in agreement with previous smaller studies.\textsuperscript{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 under-reported treatment reduction of patients with DS-ALL contributes to the increased relapse risk.\textsuperscript{48} This finding suggests that the currently accepted strategy of treatment reduction in DS-AML, which is characterized by a chemotherapy-sensitive phenotype,\textsuperscript{49} is not applicable to DS-ALL.\textsuperscript{57} The only exception may be DS-ALL patients with \textit{ETV6-RUNX1} or HeH, in which TRM outweighed the risk of relapse, for whom a 3-drug induction and a limited re-induction might be adequate. Interestingly and in accordance with previous results, the incidence of secondary malignancies was significantly lower in DS patients as compared to non-DS ALL patients. This is in agreement with the reduced propensity for solid tumors in DS patients reported before.\textsuperscript{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 \textit{CRLF2}, which is often associated with JAK-STAT mutations. In contrast to some studies showing deleterious effects of \textit{CRLF2} alterations in non-DS high-risk ALL,\textsuperscript{26,51} no such association was found in this study, nor in several prior smaller studies of DS-ALL.\textsuperscript{3,4,21,27} Nevertheless, a substantial proportion of DS-ALL patients carry these aberrations, thereby providing a pathway which might be targeted by inhibitors of the JAK-STAT pathway or mTOR signaling.\textsuperscript{52}

\textit{IKZF1} mutational status was unknown in our dataset. Recently it was shown that this gene was frequently deleted in DS-ALL patients (in \textasciitilde35\%), and was found to be an independent predictor for dismal outcome.\textsuperscript{27} Of note, the median age of patients with \textit{IKZF1} aberrations in the DS-ALL study
was significantly higher compared to wildtype patients (8.2 vs. 4.3 years), which could be an important genetic factor underlying the biological basis for the age cut-off point of 6 years reported here as clinically significant.

Previous studies reported increased TRM in children with DS-ALL, also in relapse protocols. 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 hypo-gammaglobulinemia, and hence to a higher infection rate in already immune-compromised DS patients. To reduce TRM, we suggest improving supportive care throughout the treatment period with aggressive treatment of infections, and studies analyzing the potential benefit of anti-bacterial and anti-fungal prophylaxis, and/or immunoglobulin substitution. Patients should be leucocyte 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. As 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.
Acknowledgements

We are indebted to all members of the study groups clinical centers and laboratories for contribution of data. In addition, this work was supported by KiKa (Erasmus MC - Sophia children’s hospital), and in part by grants to the Children’s Oncology Group including CA98543, U10CA98413, U24CA114766; Ergen Family Chair in Pediatric Cancer (SPH); Women’s Auxiliary Millennium Chair (JAW), NCI CA 21765 (SJCRH), the American Lebanese Syrian Associated Charities (SJCRH), the National Cancer Institute grant 5 PO1CA068484 (DFCI, LBS), and the Israel Cancer Association and Israel Science Foundation Legacy program, Waxman and Israel Cancer Research Foundations (SI).

Authorship contribution

Conception, design and writing the manuscript: T. D. Buitenkamp, S. Izraeli, J. Whitlock, C. H. Pui, C. M. Zwaan.


Conflict of interest statements: The authors declare no potential conflicts of interests.
References


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

<table>
<thead>
<tr>
<th></th>
<th>DS-ALL</th>
<th>non-DS ALL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>653</td>
<td>4445</td>
<td></td>
</tr>
<tr>
<td><strong>Age at diagnosis (range)</strong></td>
<td>5.0 (1.2-17.9)</td>
<td>4.7 (0.1-17.9)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>343</td>
<td>2431</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>310</td>
<td>2014</td>
<td></td>
</tr>
<tr>
<td><strong>Median initial WBC x 10^9/L (range)</strong></td>
<td>10.5 (0.2-459)</td>
<td>8.8 (0.2-999)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Extra medullary disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS (%)</td>
<td>16/624* (2.5)</td>
<td>98/4258* (2.2)</td>
<td>0.69</td>
</tr>
<tr>
<td>Lymphnodes (%)</td>
<td>134/412* (32.5)</td>
<td>1471/4339* (33.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Hepatomegaly (%)</td>
<td>245/469* (52.2)</td>
<td>3156/4357* (71)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testis (%)</td>
<td>1/296* (&lt;1%)</td>
<td>28/4317 (&lt;1%)</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Cytogenetic subgroups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>179/444* (40.3)</td>
<td>45/650* (6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BCR-ABL1 t(9;22)</td>
<td>3/444* (0.7)</td>
<td>93/3898* (2.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>MLL (11q23)</td>
<td>2/444* (0.5)</td>
<td>36/2966* (1.2)</td>
<td>0.15</td>
</tr>
<tr>
<td>ETV6-RUNX1 t(12;21)</td>
<td>37/444* (8.3)</td>
<td>841/3264* (25.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HeH^5</td>
<td>40/444* (9)</td>
<td>235/708* (33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HeH trisomy 4 &amp; 10</td>
<td>18 (4.5)</td>
<td>100 (42.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HeH, other</td>
<td>22 (5.5)</td>
<td>135 (57.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Others</td>
<td>183 (41.2)</td>
<td>225/650* (34.6)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>8-year OS</strong></td>
<td>74 ± 2%</td>
<td>89 ± 2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>8-year EFS</strong></td>
<td>64 ± 2%</td>
<td>81 ± 2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>8-year CIR</strong></td>
<td>26 ± 2%</td>
<td>15 ± 1%</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>2-year TRM</strong></td>
<td>7 ± 1%</td>
<td>2 ± &lt;1%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BCP, B-cell precursor; WBC, white blood cell count; CNS, central nervous system involvement at diagnosis (>5 WBC/μl; CNS-3); HeH^5 DS: 52-60 chromosomes, non-DS 51-60 chromosomes; OS, overall survival; EFS, event-free survival; TRM, treatment-related mortality; CIR, cumulative incidence of relapse, *Number of patients available for analysis.
Table 2. Contingency table representing outcome of Down syndrome patients by NCI risk group and PdL risk group criteria.

<table>
<thead>
<tr>
<th>Class</th>
<th>NCI criteria</th>
<th>PdL Risk Criteria</th>
<th>Standard risk</th>
<th>High risk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N=187</td>
<td>N=246</td>
<td>N=651</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EFS 78±3%</td>
<td>EFS 63±4%</td>
<td>NCI SR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OS 87±3%</td>
<td>OS 73±3%</td>
<td>N=433</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TRM 3±1%</td>
<td>TRM 7±2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CIR 17±3%</td>
<td>CIR 30±4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low risk</td>
<td></td>
<td>N=218</td>
<td>N=57±4%</td>
<td>NCI HR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OS 62±4%</td>
<td></td>
<td>N=218</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TRM 12±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CIR 29±3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td></td>
<td>N=0</td>
<td>EFS 57±4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OS 62±4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TRM 12±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CIR 29±3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PdL SR model</td>
<td></td>
<td>N=187</td>
<td>PdL HR model</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N=464</td>
<td></td>
<td>N=651</td>
</tr>
</tbody>
</table>

EFS, event-free survival; OS, overall survival; TRM, treatment-related mortality; CIR, cumulative incidence of relapse; SR, standard risk; HR, high risk; PdL, Ponte di Legno; Classical NCI risk criteria, age 1-9 or ≥10 years at diagnosis and WBC < or ≥50x10⁹/L; Ponte di Legno low risk criteria; age <6 years and WBC<10x10⁹/L, Ponte di Legno high risk criteria; all other patients.
Table 3. Multivariate analysis of the DS-ALL dataset

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Variable</th>
<th>HR</th>
<th>95%CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFS</td>
<td>Age &lt; 6 years</td>
<td>0.58</td>
<td>0.41 - 0.81</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>WBC &lt; 10 x 10^9/L</td>
<td>0.60</td>
<td>0.42 - 0.86</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>ETV6-RUNX1</td>
<td>0.14</td>
<td>0.03 - 0.57</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>HeH</td>
<td>0.68</td>
<td>0.34 - 1.36</td>
<td>0.275</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td>0.66</td>
<td>0.44 - 0.99</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>WBC &lt; 10 x 10^9/L</td>
<td>0.51</td>
<td>0.33 - 0.79</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>ETV6-RUNX1</td>
<td>0.12</td>
<td>0.02 - 0.86</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>HeH</td>
<td>1.01</td>
<td>0.48 - 2.11</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>RFS</td>
<td>0.48</td>
<td>0.32 - 0.73</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>WBC &lt; 10 x 10^9/L</td>
<td>0.71</td>
<td>0.46 - 1.08</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>ETV6-RUNX1</td>
<td>0.10</td>
<td>0.01 - 0.64</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>HeH</td>
<td>0.29</td>
<td>0.09 - 0.92</td>
<td>0.036</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; EFS, event free survival; OS, overall survival; RFS, relapse-free survival; WBC, white blood-cell count; HeH, high hyperdiploid (≥52 chromosomes).
Figure legends

**Figure 1. Treatment outcome of the DS-ALL and non-DS ALL patients**

The continuous lines represent the DS-ALL patients, the dotted lines represent the non-DS ALL patients. The red line represents overall survival, the blue line event free survival, the green line the cumulative incidence of treatment-related mortality and the light blue line the cumulative incidence of relapse.

**Figure 2. Treatment outcome according to age and WBC in DS-ALL.**

The overall survival (A), event-free survival (B), cumulative incidence of treatment-related mortality (C) and cumulative incidence of relapse (D) are depicted for patients with age <6 years and WBC <10 x 10^9/L (blue line) versus all other DS-ALL patients (red line). The numbers on the curves for overall survival and event-free survival represent results at 8 years. The numbers on the curves for treatment-related mortality are 2-year results (during treatment only) and those for relapse are results at 8 years.
Figure 1.

![Graph showing survival analysis results.](image)

<table>
<thead>
<tr>
<th>Survival non-DS (N=4445, 504 events)</th>
<th>Survival DS (N=653, 153 events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFS non-DS (N=4445, 840 events)</td>
<td>EFS DS (N=653, 212 events)</td>
</tr>
<tr>
<td>CIR non-DS (N=650)</td>
<td>CIR DS (N=148)</td>
</tr>
<tr>
<td>TRM non-DS (N=87)</td>
<td>TRM DS (N=50)</td>
</tr>
</tbody>
</table>

\[ p<0.0001 \]
Figure 2

A)  
B)  
C)  
D)
Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group


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