Minimal residual disease, long-term outcome, and *IKZF1* deletions in children and adolescents with Down syndrome and acute lymphocytic leukaemia: a matched cohort study

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Summary

Background Patients with Down syndrome and acute lymphocytic leukaemia are at an increased risk of treatment-related mortality and relapse, which is influenced by unfavourable genetic aberrations (eg, *IKZF1* deletion). We aimed to investigate the potential underlying effect of Down syndrome versus the effects of adverse cancer genetics on clinical outcome.

Method Patients (aged 1–23 years) with Down syndrome and acute lymphocytic leukaemia and matched non-Down syndrome patients with acute lymphocytic leukaemia (matched controls) from eight trials (DCOG ALL10 and ALL11, ANZCHOG ALL8, AIEOP-BFM ALL2009, UKALL2003, NOPHO ALL2008, CoALL 07-03, and CoALL 08-09) done between 2002 and 2018 across various countries (the Netherlands, the UK, Australia, Denmark, Finland, Iceland, Norway, Sweden, and Germany) were included. Participants were matched (1:3) for clinical risk factors and genetics, including *IKZF1* deletion. The primary endpoint was the comparison of MRD levels (absolute MRD levels were categorised into two groups, low [<0·0001] and high [≥0·0001]) between patients with Down syndrome and acute lymphocytic leukaemia and matched controls, and the secondary outcomes were comparison of long-term outcomes (event-free survival, overall survival, relapse, and treatment-related mortality [TRM]) between patients with Down syndrome and acute lymphocytic leukaemia and matched controls. Two matched cohorts were formed: for MRD analyses and for long-term outcome analyses. For both cohorts, matching was based on induction regimen; for the long-term outcome cohort, matching also included MRD-guided treatment group. We used mixed-effect models, Cox models, and competing risk for statistical analyses.

Findings Of 251 children and adolescents with Down syndrome and acute lymphocytic leukaemia, 136 were eligible for analyses and matched to 407 (of 8426) non-Down syndrome patients with acute lymphocytic leukaemia (matched controls). 113 patients with Down syndrome and acute lymphocytic leukaemia were excluded from matching in accordance with predefined rules, no match was available for two patients with Down syndrome and acute lymphocytic leukaemia. The proportion of patients with high MRD at the end of induction treatment was similar for patients with Down syndrome and acute lymphocytic leukaemia (52 [38%] of 136) and matched controls (157 [39%] of 403; OR 0·97 [95% CI 0·64–1·46]; p=0·88). Patients with Down syndrome and acute lymphocytic leukaemia had a higher relapse risk than did matched controls in the *IKZF1* deleted group (relapse at 5 years 37·1% [17·1–57·2] *vs* 13·2% [6·1–23·1]; cause-specific hazard ratio [HR_{cs}] 4·3 [1·6–11·0]; p=0·0028), but not in the *IKZF1* wild-type group (relapse at 5 years 5·8% [2·1–12·2] *vs* 8·1% [5·1–12·0]; HR_{cs} 1·0 [0·5–2·1]; p=0·99). In addition to increased induction deaths (15 [6%] of 251 *vs* 69 [0·8%] of 8426), Down syndrome and acute lymphocytic leukaemia was associated with a higher risk of post-induction TRM compared with matched controls (TRM at 5 years 12·2% [7·0–18·9] *vs* 2·7% [1·3–4·9]; HR_{cs} 5·0 [2·3–10·8]; p<0·0001).

Interpretation Induction treatment is equivalently effective for patients with Down syndrome and acute lymphocytic leukaemia and for matched patients without Down syndrome. Down syndrome itself provides an additional risk in individuals with *IKZF1* deletions, suggesting an interplay between the germline environment and this poor risk somatic aberration. Different treatment strategies are warranted considering both inherent risk of relapse and high risk of TRM.

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Research in context

Evidence before this study

We did not do a formal search of the literature before starting this study. Compared with patients without Down syndrome and acute lymphocytic leukaemia, the clinical outcome of patients with Down syndrome and acute lymphocytic leukaemia is reported to be inferior due to an increased risk of relapse and increased acute and long-term treatment-related toxicity. Nevertheless, sample sizes within national studies remain small due to the rare nature of this group of patients. In a study by Buitenkamp and colleagues published in 2014, results were reported of a large multinational effort to study the clinical outcome of patients with Down syndrome. However, this study included data collected mostly before minimal residual disease (MRD) was used as a predictor of acute lymphocytic leukaemia relapse; therefore, did not evaluate this well known prognostic factor. Other studies on Down syndrome and acute lymphocytic leukaemia have addressed the distinct genetic features, such as the lower frequency of the good prognostic lesions *ETV6-RUNX1* and high hyperdiploidy, and the higher frequency of the poor risk *IKZF1* deletion than in patients without Down syndrome. Previous studies comparing acute lymphocytic leukaemia in individuals with and without Down syndrome were not matched for genetic features and were, therefore, unable to analyse whether the poor risk of children and adolescents with Down syndrome was due to a higher frequency of high risk genetics, or whether Down syndrome itself negatively affects clinical outcome in matched genetic subgroups. Within the collaborative parties of the Ponte di Legno group, no similar project had been started previously and, to the best of our knowledge, no papers have addressed these questions before.

Added value of this study

Our matched cohort study is the first, to our knowledge, multinational study to address both MRD and long-term clinical outcome in Down syndrome and non-Down syndrome patients with acute lymphocytic leukaemia, matched according to clinical risk factors and genetics. We show that MRD levels at the end of induction are similar between individuals with and without Down syndrome. Our findings also show that Down syndrome itself provides an additional risk of relapse in patients with *IKZF1* deletion, which suggests an interplay between the poor risk of *IKZF1* deletion and the germline environment of children with Down syndrome.

Implications of all the available evidence

Our study emphasises the need for separate risk group stratification and treatment strategies for individuals with Down syndrome and acute lymphocytic leukaemia. The results will help to identify individuals with Down syndrome who are at the highest risk of relapse of acute lymphocytic leukaemia and, therefore, highlight for which patients treatment intensification is warranted. However, such changes should be carefully balanced against the risk of treatment-related mortality.

Introduction

Acute lymphocytic leukaemia occurs more frequently in children with Down syndrome than in those without.[1] Patients with Down syndome and acute lymphocytic leukaemia are at a higher risk of relapse and treatment-related mortality (TRM) than non-Down syndrome patients with acute lymphocytic leukaemia.[2,3] The increased risk of relapse in patients with Down syndrome and acute lymphocytic leukaemia might be linked to adverse cancer genetics,[2,3] insufficient treatment adherence,[4] and an increased risk of fatal treatment complications. The lesions *ETV6-RUNX1* and high hyperdiploidy with improved prognosis are less frequent in patients with Down syndrome and acute lymphocytic leukaemia than in non-Down syndrome patients with acute lymphocytic leukaemia (8% *vs* 25% and 9% *vs* 33%, respectively).[2,3] *IKZF1* deletions are more frequent in patients with Down syndrome and acute lymphocytic leukaemia than in non-Down syndrome patients with acute lymphocytic leukaemia (35% *vs* 17%)[3,5,6] and are associated with poor clinical outcome.[3,5]

Minimal residual disease (MRD) is monitored by real-time quantitative PCR[7] or flow cytometry[8] at fixed time points during acute lymphocytic leukaemia treatment. MRD is the most powerful prognostic factor associated with the incidence of relapse in children with newly diagnosed acute lymphocytic leukaemia[9] and is, therefore, included in risk group stratification in contemporary acute lymphocytic leukaemia treatment protocols. High MRD levels after induction therapy show that a more intensive therapy regimen should be given to reduce risk of relapse.[10–12] Low MRD levels suggest that low intensity therapy is probably sufficient to avoid relapse and is preferable to reduce unnecessary acute and long-term side effects of treatment.[10,13] A previous study within the UKALL2003 cohort showed that MRD levels during acute lymphocytic leukaemia treatment can discriminate between patients with Down syndrome and acute lymphocytic leukaemia at high risk of relapse.[3]

Previous research has mainly compared patients with Down syndrome and acute lymphocytic leukaemia with non-Down syndrome patients with acute lymphocytic leukaemia, which are biased by differences in the distribution of genetic aberrations, especially a higher incidence of *IKZF1* deletion in patients with Down syndrome and acute lymphocytic leukaemia. We aimed to investigate the underlying effect of Down syndrome on MRD levels and long-term clinical outcome in a multinational cohort of children and adolescents with Down syndrome and acute lymphocytic leukaemia and matched non-Down syndrome controls from eight MRD-guided trials. We matched participants for clinical risk

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factors and genetic aberrations, including *IKZF1* deletion.

Methods

Study design and participants

In this matched cohort study, we enrolled children and adolescents (aged 1–23 years) with Down syndrome and acute lymphocytic leukaemia and non-Down syndrome children with acute lymphocytic leukaemia (referred to as matched controls) who were treated between 2002 and 2018 in two consecutive Dutch Childhood Oncology Group trials (DCOG ALL10[10] and ALL11), two Australian trials (ANZCHOG ALL8[12,14] and AIEOP-BFM ALL2009), one UK trial (UKALL2003[11,13]), one trial from the Nordic Society of Pediatric Hematology-Oncology (NOPHO ALL2008[15]), and two trials from the Co-Operative Study Group for Childhood Acute Lymphocytic Leukemia in Germany (CoALL 07-03[16] and CoALL 08-09). Only trials with MRD-guided protocols were selected. Each collaborative group arranged medical research ethics committee approval and informed consent from patients, parents, or guardians, according to local law and regulations. Patient features and clinical outcome parameters were collected as part of each trial, centrally compiled by the respective study group, and shared with the investigators at the Princess Máxima Center for Pediatric Oncology for the purpose of this study. Using these data, we did a retrospective cohort study.

Procedures

We combined previously reported data of patients with Down syndrome and acute lymphocytic leukaemia[2,3,5,17] with new, unpublished data compiled in a matched design. Drugs administered during induction treatment in every protocol, including the adjustments made for patients with Down syndrome and acute lymphocytic leukaemia, are described in the appendix (p 5). Patients with Down syndrome and acute lymphocytic leukaemia were not stratified to the high-risk treatment groups in the Dutch trials (DCOG ALL10 and ALL11), and not to the standard risk treatment group in the NOPHO trial (appendix pp 6–8). For two of the included patients with Down syndrome and acute lymphocytic leukaemia, treatment was adjusted because of Down syndrome status, both cases were assigned to a lower risk group, one with *IKZF1* deletion, but neither suffered from a relapse.

See Online for appendix

IKZF1 deletion and the major cytogenetic subtypes—*ETV6-RUNX1*, *TCF3-PBX1*, *KMT2A*/*MLL*-rearranged, *BCR-ABL1*, and high hyperdiploidy (51–65 chromosomes)—were determined by the collaborative study group reference laboratories. Patients negative or not tested for the major cytogenetic lesions were classified as other B-lineage acute lymphocytic leukaemia. *IKZF1* deletions were identified with multiplex ligation-dependent probe amplification assays (SALSA P335 ALL-*IKZF1* or SALSA P202 *IKZF1*, or both; MRC-Holland, Amsterdam, Netherlands), according to the manufacturer's protocol.

MRD was measured with real-time quantitative PCR in the DCOG, UK, Australian, and CoALL trials and with flow cytometry in the NOPHO trial. The DCOG, UK, NOPHO, and CoALL trials have been analysed together previously with respect to MRD.[18] The sensitivity of both methods is sufficient to quantify MRD levels of at least 0·0001,[19,20] making this a reliable cutoff when analysing MRD levels as categorical outcome variable.

Outcomes

The primary endpoint was MRD levels between patients with Down syndrome and acute lymphocytic leukaemia and matched controls. MRD was measured at the end of induction treatment for all trials, and additionally at the end of consolidation treatment for the DCOG and Australian trials.

The secondary endpoint was long-term outcomes (event-free survival, overall survival, relapse, and TRM) in patients with Down syndrome and acute lymphocytic leukaemia and matched controls. Complete remission was defined as less than 5% leukaemic cells in the bone marrow and recovery of normal haematopoiesis, and the absence of leukaemia elsewhere. Induction death was defined as death before MRD-guided risk group stratification. Early death was defined as death before complete remission. All other participants in our cohort reached complete remission. Relapse was defined by disease recurrence after initial complete remission and TRM was defined as any death in first complete remission. Event-free survival was defined as the time from diagnosis to relapse, second malignancy, or death, whichever happened first. Overall survival was defined as the time from diagnosis to death from any cause.

Statistical analyses

Induction deaths, patients with missing data on *IKZF1* status, and patients with no MRD data available were excluded from matching. Each patient with Down syndrome and acute lymphocytic leukaemia was matched to three non-Down syndrome patients with acute lymphocytic leukaemia (ie, matched controls). Matching was done according to type of induction treatment, cytogenetic subtype (*BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, *MLL* rearranged, high hyperdiploid, and other B-lineage), *IKZF1* status (deleted *vs* not deleted), age at diagnosis (<10 *vs* 10–23 years), and white blood cell count at diagnosis ($<50\times10^9$ *vs* $\geq50\times10^9$ cells per L). The date of diagnosis of matched controls was as close as possible to the date of diagnosis of acute lymphocytic leukaemia in patients with Down syndrome to avoid bias in patient inclusion, treatment, or supportive care changes. Only if the number of non-Down syndrome patients with acute lymphocytic leukaemia available was too few to provide matched controls for multiple patients with Down syndrome and acute lymphocytic leukaemia, could a

non-Down syndrome and acute lymphocytic leukaemia control act as a matched control for more than one patient with Down syndrome and acute lymphocytic leukaemia (ie, matching with replacement). If fewer than three non-Down syndrome and acute lymphocytic leukaemia controls were available for one patient with Down syndrome and acute lymphocytic leukaemia, the number of controls was restricted to two or one, which was compatible with all the statistical models used in this study. If no matched controls were available, the patient with Down syndrome and acute lymphocytic leukaemia was excluded. For MRD analyses, matching was based on induction treatment. For long-term outcome analyses, matching was based on induction treatment and MRD-guided treatment group. Matching on induction treatment required induction regimens to match but

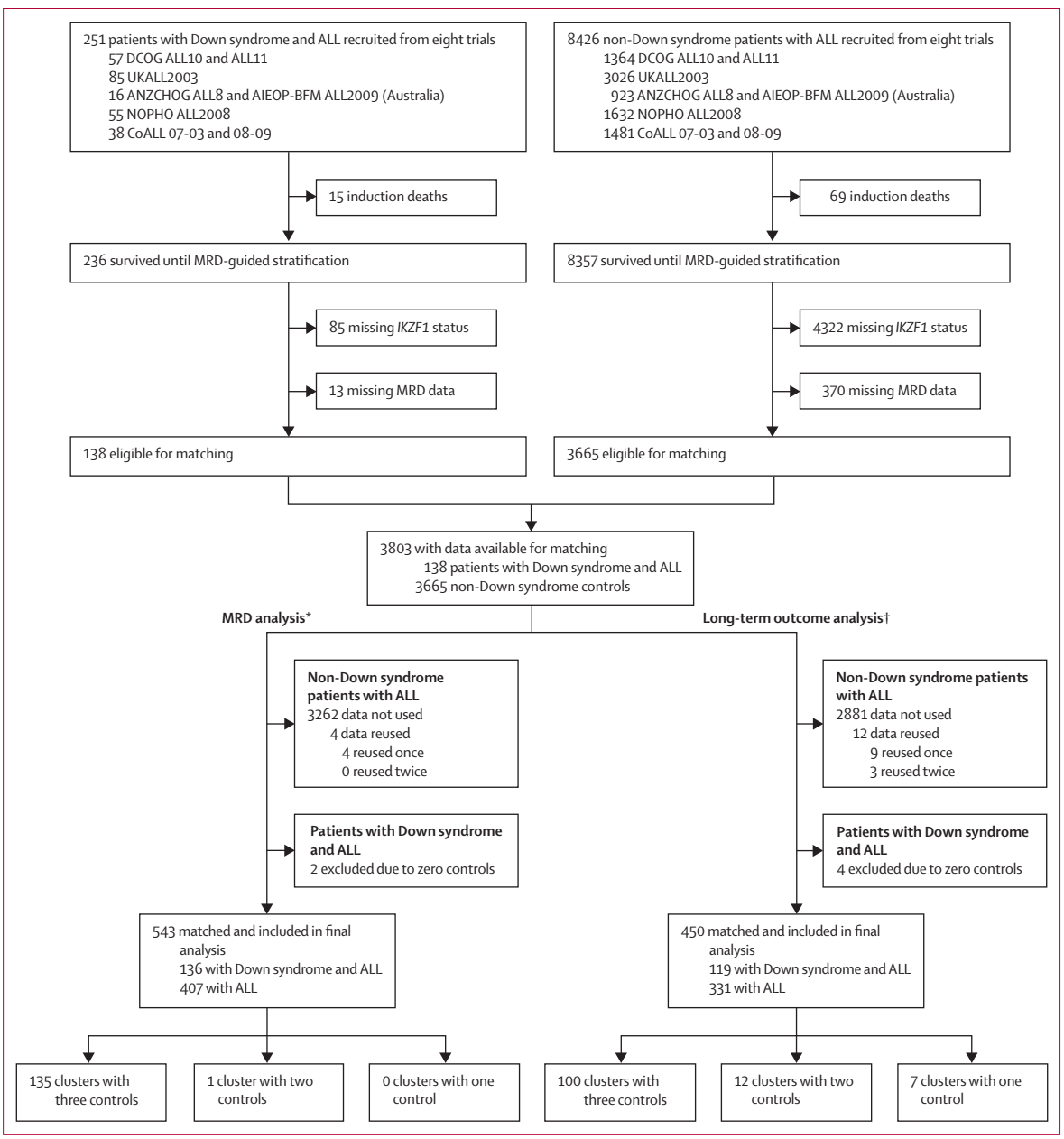


Figure 1: **Study profile**
Patients with zero controls were excluded from analyses. ALL=acute lymphocytic leukaemia. ANZCHOG=The Australian and New Zealand Children's Haematology and Oncology Group. AIEOP-BFM = Associazione Italiana Ematologia Oncologia Pediatrica-Berlin-Frankfurt-Münster. DCOG=Dutch Childhood Oncology Group. NOPHO=Nordic Society of Pediatric Hematology-Oncology. CoALL=Co-Operative Study Group for Childhood Acute Lymphoblastic Leukemia. MRD=minimal residual disease. *Matching not based on MRD-guided treatment group. †Matching based on MRD-guided treatment group. Long-term outcome data were unavailable for 15 patients with Down syndrome and ALL and for 468 non-Down syndrome patients with ALL.

doses did not have to be exactly the same. Patients who did not have survival data available were excluded before matching. Therefore, our study used two separately matched cohorts of patients with Down syndrome and acute lymphocytic leukaemia and non-Down syndrome and acute lymphocytic leukaemia matched controls (figure 1).

Absolute MRD levels were categorised into two groups (low [<0·0001] and high [≥0·0001]) and analysed in a mixed-effects cumulative logit model for ordinal response data. Matching was included as random effect in the mixed-effects models. Odd ratios (ORs) show the effect of groups on MRD; ORs were computed using the exponential function of the estimate from the mixed model. Data on patients who had not had an event before the end of follow-up were censored on the date of last follow-up. We calculated estimates of the probability of survival using the Kaplan-Meier method. Hazard ratios (HRs) for the effect of groups on survival were estimated by a Cox proportional hazards regression model. To estimate the cumulative incidence of relapse and TRM, a competing risk model was used. A cause-specific Cox proportional hazards regression model was estimated to assess the effect of groups on the two competing events of relapse and TRM. Patients who reached complete remission were included in the competing risk analyses, excluding early deaths. Interactions were tested by evaluating the significance of cross-product terms. After the Cox model, a linear combination of regression parameters was computed to make the comparisons for all separate groups in the Down syndrome and *IKZF1* model. Study group was incorporated as a stratification variable in the Cox models. Matching was included as cluster variable in the Cox models. Schoenfeld residuals were used to test the proportional hazards assumption in the Cox models and

	MRD cohort			Long-term outcome cohort		
	Patients with Down syndrome and acute lymphocytic leukaemia (n=136)	Non-Down syndrome patients with acute lymphocytic leukaemia (n=407)	p value	Patients with Down syndrome and acute lymphocytic leukaemia (n=119)	Non-Down syndrome patients with acute lymphocytic leukaemia (n=331)	p value
Cytogenetics*						
ETV6-RUNX1	22 (16%)	66 (16%)	NA	19 (16%)	55 (17%)	NA
BCR-ABL1	0	0	..	0	0	..
KMT2A rearranged	0	0	..	0	0	..
TCF3-PBX1	0	0	..	0	0	..
High hyperdiploid	6 (4%)	15 (4%)	..	6 (5%)	15 (5%)	..
Other B-lineage	109 (80%)	326 (80%)	..	95 (80%)	261 (79%)	..
IKZF1* deletion						
Yes	26 (19%)	78 (19%)	NA	24 (20%)	65 (20%)	NA
No	110 (81%)	329 (81%)	..	95 (80%)	266 (80%)	..
Age at diagnosis, years*						
<10	102 (75%)	306 (75%)	NA	91 (76%)	254 (77%)	NA
≥10	34 (25%)	101 (25%)	..	28 (24%)	77 (23%)	..
Median (IQR)†	4·8 (3·2–9·7)	5·0 (2·8–9·9)	NA	4·1 (3·0–8·2)	4·8 (3·0–8·9)	NA
White blood cell count at diagnosis, cells per L*						
$<50\times10^9$	113 (83%)	339 (83%)	NA	98 (82%)	275 (83%)	NA
$\geq50\times10^9$	23 (17%)	68 (17%)	..	21 (18%)	56 (17%)	..
MRD-guided risk group stratification*						
Low	NA	NA	NA	37 (31%)	110 (33%)	NA
Intermediate	NA	NA	..	62 (52%)	164 (50%)	..
High	NA	NA	..	20 (17%)	57 (17%)	..
Sex						
Male	63/108 (58%)	178/324 (55%)	0·61	61/106 (58%)	168/310 (54%)	0·63
Female	45/108 (42%)	146/324 (45%)	..	45/106 (42%)	142/310 (46%)	..
CNS involvement						
Yes	0/67	3/201 (1%)	0·74	0/66	1/194 (1%)	1·00
No	67/67 (100%)	198/201 (99%)	..	66/66 (100%)	193/194 (99%)	..

Unless otherwise stated, data are n (%) or n/N (%) if data were not available for all patients. χ^2 test was used to calculate p values. Data on ethnicity were not available. MRD=minimal residual disease. NA=not applicable.*No statistical test was done because patients were matched on these criteria. †Data were available for 483 of 543 participants in the MRD cohort and 244 of 450 participants in the long-term outcome cohort.

Table: **Patient characteristics**

all models passed. We report estimated ORs, HRs, survival rates, and cumulative incidences, with their 95% CIs. All 95% CIs were calculated within each respective model. We used the χ^2 test *to* calculate p values for patient characteristics. Two-sided p values of 0·05 or less were considered significant. We used R (version 3.6.3) for all statistical analyses.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From eight trials, 251 patients with Down syndrome and acute lymphocytic leukaemia and 8426 non-Down syndrome patients with acute lymphocytic leukaemia (matched controls) were enrolled to this cohort study. During induction treatment, 15 (6%) of 251 patients with Down syndrome and acute lymphocytic leukaemia and 69 (0·8%) of 8426 matched controls died. Induction deaths were excluded from matching; therefore, we report post-induction TRM. 85 patients were excluded for missing data on *IKZF1* deletion and 13 for lack of MRD data. Matching was done for 136 patients with Down syndrome and acute lymphocytic leukaemia to study differences in MRD levels, and for 119 patients with Down syndrome and acute lymphocytic leukaemia to analyse long-term clinical outcome (figure 1). Matching with replacement was done for 12 patients in the long-term outcome cohort and for four patients in the MRD cohort. In the matched cohort used for MRD analyses, 28 (21%) of the 136 patients with Down syndrome and acute lymphocytic leukaemia cases and their matched controls had favourable cytogenetics (*ETV6-RUNX1* or high hyperdiploid), 26 (19%) had *IKZF1* deletion, 34 (25%) were 10 years or older at diagnosis, and 23 (17%) had a white blood cell count of at least 50×10^9 cells per L (table; appendix p 9). These percentages were similar in the matched cohort used for long-term outcome analyses, in which 20 (17%) of 119 were treated according to a high-risk treatment group (table; appendix p 10). A comparison between the included and excluded patients with Down syndrome and acute lymphocytic leukaemia is shown in the appendix (p 11).

In addition to 13 excluded patients with no MRD data (figure 1), four matched controls had no MRD data at end of induction. Therefore, for 136 patients with Down syndrome and acute lymphocytic leukaemia and 403 of 407 matched controls MRD at end of induction could be analysed. For 54 patients with Down syndrome and acute lymphocytic leukaemia and 168 matched controls MRD at end of consolidation could be analysed; for other patients MRD was not measured at end of consolidation. The percentage of patients in the higher MRD category did not differ between patients with Down syndrome and acute lymphocytic leukaemia versus

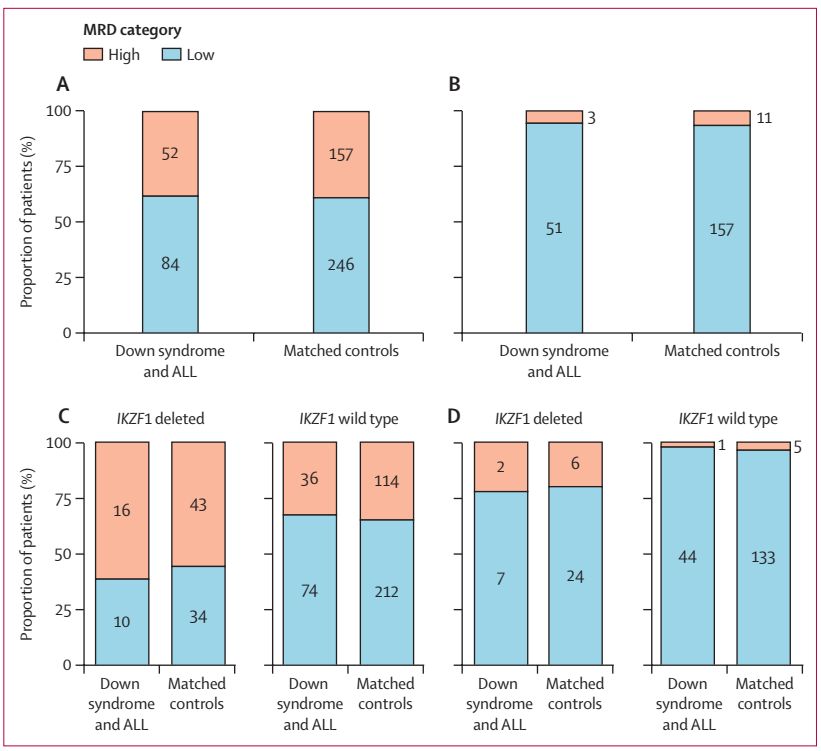


Figure 2: **Proportion of participants by MRD category, matched for risk factors**
Data are for all participants with available data at the end of induction treatment (A) and the end of consolidation treatment (B), at the end of induction in the *IKZF1* deleted and *IKZF1* wild-type group (C), and at the end of consolidation in the *IKZF1* deleted and *IKZF1* wild-type group (D). Matched controls are non-Down syndrome patients with ALL. Numbers within plots show the number of patients in each MRD category (low MRD <0·0001; high MRD ≥0·0001). MRD=minimal residual disease.

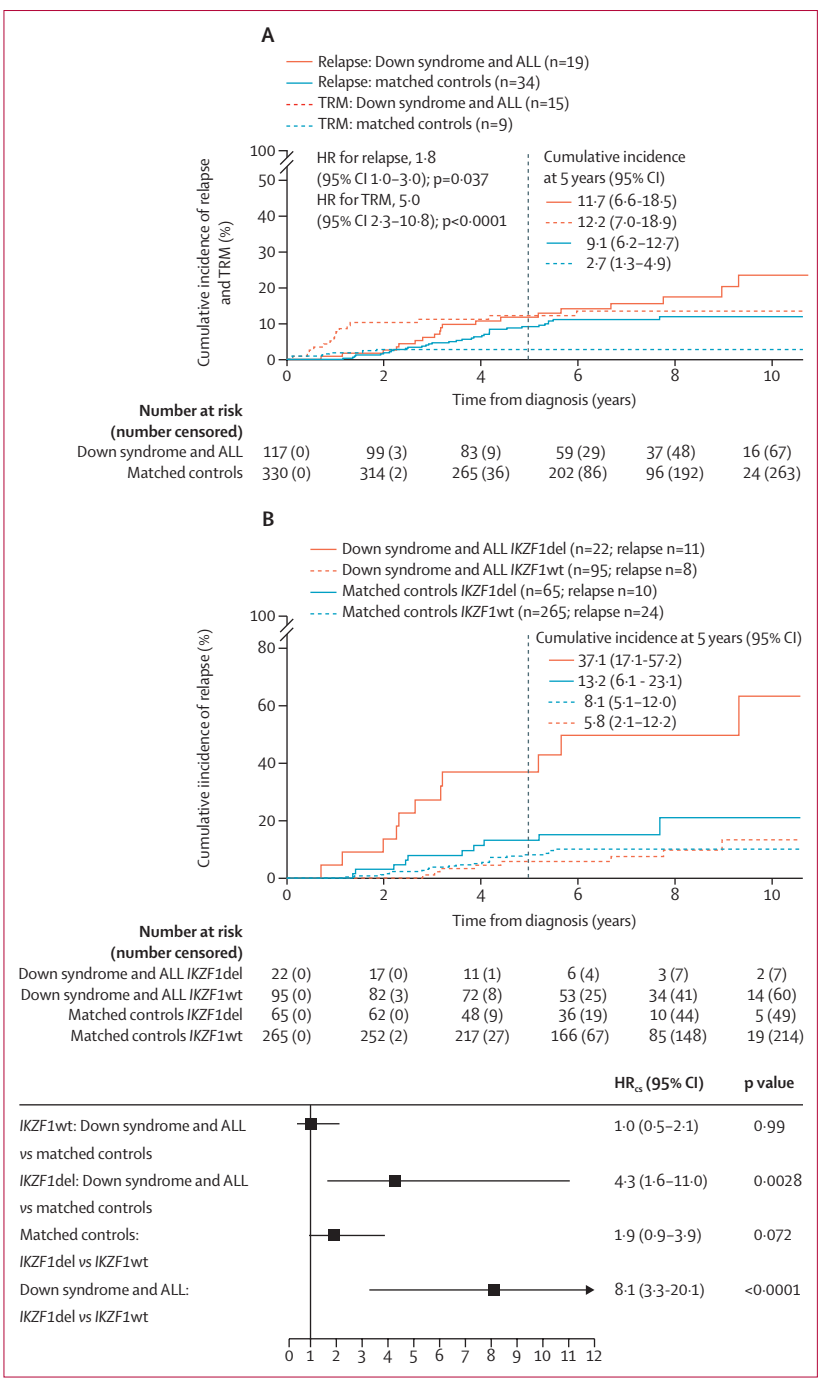
matched controls at end of induction (38% [52/136] *vs* 39% [157/403]; OR 0·97 [95% CI 0·64–1·46]; p=0·88; figure 2A) nor at end of consolidation (6% [3/54] *vs* 7% [11/168]; OR 0·80 [0·19–3·34]; p=0·76; figure 2B). In patients with and without *IKZF1* deletion, the percentage of patients in the higher MRD category was similar for patients with Down syndrome and acute lymphocytic leukaemia and matched controls at both end of induction (*IKZF1* deleted: 62% [16/26] *vs* 56% [43/77]; *IKZF1* wildtype: 33% [36/110] *vs* (35% [114/326]; OR 0·97 [0·64–1·46]; p=0·88; figure 2C) and at end of consolidation (*IKZF1* deleted: 22% [2/9] *vs* 20% [6/30]; *IKZF1* wildtype: 2% [1/45] *vs* 4% [5/138]; OR 0·83 [0·20–3·45]; p=0·79; figure 2D). Median follow-up time was 7·2 years (IQR 5·5–8·8), on the basis of the reverse Kaplan-Meier method. Event-free survival was worse for patients with Down syndrome and acute lymphocytic leukaemia (n=119) than for matched controls (n=331; HR 2·5 [95% CI 1·6–3·9]; p<0·0001; appendix p 3), even for *ETV6-RUNX1*-positive participants (event-free survival at 5 years 79% [62–100] *vs* 96% [91–100]; HR 10·7 [2·7–42·5]; p=0·00080; appendix p 4). Overall survival was also worse worse for patients with Down syndrome and acute lymphocytic leukaemia than for matched controls (HR 3·8 [2·2–6·3]; p<0·0001). 5-year event-free survival

was 75% (95% CI 67–83) in patients with Down syndrome and acute lymphocytic leukaemia compared with 88% (84–92) in matched controls, and 5-year overall survival was 77% (70–85) in patients with Down syndrome and acute lymphocytic leukaemia compared with 94% (91–97) in matched controls (appendix p 3).

MRD levels were higher in patients with Down syndrome and acute lymphocytic leukaemia and their matched controls in the UK trial (UKALL2003) than in patients enrolled in the DCOG trials, based on a mixed-effects model (OR 2·4 [95% CI 1·5–3·8]; p=0·00031; appendix p 1). Event-free survival was worse in patients with Down syndrome and acute lymphocytic leukaemia and their matched controls enrolled in the Australian trials (ANZCHOG ALL8 and AIEOP-BFM ALL2009) than in patients enrolled in the DCOG trial, based on a Cox model (HR 2·0 [1·1–3·8]; p=0·028; appendix p 2). Since study group was included as a stratification variable in the statistical models, these differences did not affect the results.

Three patients, two of whom had Down syndrome and acute lymphocytic leukaemia, died early (ie, before complete remission) and were excluded from relapse and post-induction TRM analyses. Patients with Down syndrome and acute lymphocytic leukaemia (n=117) had a higher risk of post-induction TRM than did matched controls (n=330; cause-specific HR [HR_{cs}] 5·0 [95% CI 2·3–10·8]; p<0·0001). The 5-year post-induction TRM was 12·2% (95% CI 7·0–18·9) in patients with Down syndrome and acute lymphocytic leukaemia and 2·7% (1·3–4·9) in matched controls (figure 3A). The 5-year cumulative incidence of relapse was 11·7% (6·6–18·5) in the patients with Down syndrome and acute lymphocytic leukaemia and 9·1% (6·2–12·7) in the matched controls (figure 3A). In the Down syndrome and acute lymphocytic leukaemia group, four very late relapses occurred (>6 years after diagnosis), which was only seen in one individual in the matched controls group (figure 3A). These patients did not belong to a single genetic group or share one specific known risk factor. There was a small difference in relapse risk, with a HR_{cs} for patients with Down syndrome and acute lymphocytic leukaemia compared with matched controls of 1·8 (95% CI 1·0–3·0; p=0·037; figure 3A).

By adding the interaction between *IKZF1* status and Down syndrome plus acute lymphocytic leukaemia to the model, we showed that *IKZF1* deletion was differentially associated to relapse in patients with Down syndrome and acute lymphocytic leukaemia versus matched controls (HR_{cs} 4·2 [95% CI 1·3–14·1]; p=0·018). Patients with Down syndrome and acute lymphocytic leukaemia had a higher risk of relapse than did matched controls among patients with *IKZF1* deletion (HR_{cs} 4·3 [1·6–11·0]; p=0·0028; figure 3B). Patients with Down syndrome and acute lymphoblastic leukaemia did not have a higher risk of relapse than did matched controls among patients without *IKZF1* deletion (HR_{cs} 1·0 [0·5–2·1]; p=0·99). The 5-year cumulative incidence of relapse was 37·1% (17·1–57·2) in the Down syndrome and acute lymphocytic leukaemia with *IKZF1* deletion group (n=22), 13·2% (6·1–23·1) in the matched controls with *IKZF1* deletion group (n=65), 5·8% (2·1–12·2) in the Down syndrome and acute lymphocytic leukaemia *IKZF1* wild-type group (n=95), and 8·1% (5·1–12·0) in the matched controls with *IKZF1* wild-type group (n=265).



(Figure 3 continues on next page)

Among patients with Down syndrome and acute lymphocytic leukaemia with *IKZF1* deletion, the 5-year cumulative incidence of relapse was higher in the group with high MRD levels (56·4% [95% CI 23·1–80·0]; n=13) than in the group with low MRD levels (11·1% [0·5–40·6; n=9; figure 3C). A similar trend was seen in the patients with Down syndrome and acute lymphocytic leukaemia without *IKZF1* deleted (13·5% [4·1–28·5] in the 31 patients with high MRD levels *vs* 1·6% [0·1–7·8] in the 64 patients with low MRD levels).

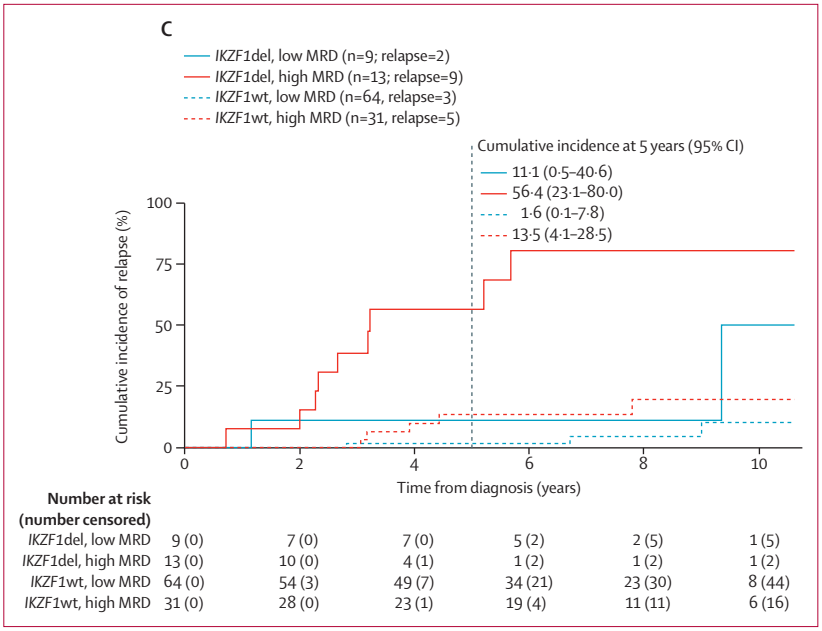


Figure 3: **Estimated cumulative incidences, matched for risk factors**
(A) Cumulative incidence of relapse and TRM in patients with Down syndrome and ALL (n=117) and matched controls (n=330). Matched controls are non-Down syndrome patients with ALL. (B) Estimated effect of Down syndrome and *IKZF1* deletions on relapse. (C) Estimated effect of MRD at end of induction treatment (low <0·0001; high ≥0·0001) on relapse in patients with Down syndrome and acute lymphocytic leukaemia with and without *IKZF1* deletion. All estimates are accompanied by 95% CIs. ALL=acute lymphocytic leukaemia. del=deleted. HR=hazard ratio. MRD=minimal residual disease. TRM=treatment-related mortality. wt=wild type.

Discussion

This matched case control study aimed to reduce bias arising from the higher prevalence of adverse risk factors in patients with Down syndrome and acute lymphocytic leukaemia compared with matched controls (non-Down syndrome patients with acute lymphocytic leukaemia). To our knowledge, this is the first study that included patients with acute lymphocytic leukaemia who did and did not have Down syndrome matched for clinical and genetic risk factors to address questions on MRD response and long-term outcome in a large, international group of children treated according to contemporary MRD-guided protocols. Our findings show that the increased risk of relapse previously assigned to patients with Down syndrome and acute lymphocytic leukaemia[2,3] is not exclusively due to a higher prevalence of adverse cancer genetics,[2,3,5,6] but could be inherent to Down syndrome. Given that this risk is only increased in *IKZF1*-deleted patients with Down syndrome and acute lymphocytic leukaemia suggests an interplay between the germline environment and this specific poor risk somatic aberration. Patients with Down syndrome and acute lymphocytic leukaemia with *IKZF1* deletion have a very poor prognosis and high MRD levels identify the patients at the highest risk of relapse (56·4% at 5 years [95% CI 23·1–80·0]). These results build upon previous studies of patients with Down syndrome and acute lymphocytic leukaemia showing the prognostic value of *IKZF1*[3,5] and MRD[3] individually, and support the inclusion of both parameters in the stratification of patients with Down syndrome and acute lymphocytic leukaemia, as is incorporated in the recently started ALLTogether1 trial (NCT03911128).

The increased risk of relapse that is inherent to Down syndrome in *IKZF1*-deleted patients might be explained partially by the reduced treatment intensity in patients with Down syndrome and acute lymphocytic leukaemia, both by different risk group allocation and protocol adjustments for patients with Down syndrome and acute lymphocytic leukaemia in general (appendix pp 6–8), and as the result of individual adjustments due to toxicity. Individual lower risk group allocation only applied to two patients with Down syndrome and acute lymphocytic leukaemia who received medium-risk treatment instead of high-risk treatment and did not suffer a relapse. Data on individual patient level adjustments were absent and could not be taken into account. We assume patients were treated with the maximum tolerated treatment intensity to prevent relapse. The increased risk of relapse could be explained by a synergy between *IKZF1* deletion and genes located at the constitutional extra copy of chromosome 21 or other frequent lesions in patients with Down syndrome and acute lymphocytic leukaemia (eg, *CRLF2* aberrations). We could not address the effect of *CRLF2* aberrations in the present study since these data were not available for most patients. About 60% of patients with Down syndrome and acute lymphocytic leukaemia[5,21,22] and 10% of non-Down syndrome patients with acute lymphocytic leukaemia have *CRLF2* aberrations.[6,21] Most multivariable analyses showed that *CRLF2* aberration is neither an independent predictor of outcome,[5,6,21] nor is it specific for patients with Down syndrome and acute lymphocytic leukaemia.[3,5,22] Targeted drugs directed against *CRLF2* downstream pathways, such as Janus kinase inhibitors, might still be a valuable treatment option to reduce the use of conventional chemotherapy.[23] A phase 2 study of the Janus kinase inhibitor ruxolitinib with chemotherapy for children with acute lymphocytic leukaemia (NCT02723994) is ongoing, but patients with Down syndrome are excluded. An alternative poor prognostic factor is the *IKZF1*^{plus} signature, which combines *IKZF1* deletion with deletion(s) in *PAX5*, the *PAR1* region, or *CDKN2A/B*, with the absence of *ERG* deletion. This signature is associated with worse outcomes than *IKZF1* deletion alone in some treatment protocols for

acute lymphocytic leukaemia,[24] and might modulate the high impact of *IKZF1* deletion in patients with Down syndrome and acute lymphocytic leukaemia. A germline single nucleotide polymorphism (SNP) in *IKZF1*, more often found in patients with acute lymphocytic leukaemia that also have Down syndrome than in those without, leads to reduced enhancer activity and DNA-protein binding, which has been associated with lower *IKZF1* expression. The *IKZF1* SNP has been linked to acute lymphocytic leukaemia susceptibility in people with Down syndrome and might have a stronger proleukaemic effect in patients with Down syndrome and acute lymphocytic leukaemia than in non-Down syndrome patients with acute lymphocytic leukaemia.[25] This hypothesis supports our findings that inactivation of *IKZF1* has a stronger unfavourable effect in patients with acute lymphocytic leukaemia that also have Down syndrome than in those without. A germline SNP of *ERG* has also been linked to the increased risk of acute lymphocytic leukaemia in children with Down syndrome.[26] The detailed genetic background of patients with Down syndrome and acute lymphocytic leukaemia was beyond the scope of this research, but future research on these genetic factors is warranted.

Our study has a few limitations. Despite this large international collaboration, the number of patients with Down syndrome and acute lymphocytic leukaemia with *IKZF1* deletion in the long-term outcome analysis was small (n=22), and further studies in a larger patient cohort where *IKZF1* status is collected prospectively are needed. Another limitation is the absence of sufficient MRD data at end of consolidation to analyse the correlation with relapse.

Within our matched cohort, we showed that even in *ETV6-RUNX1* translocated leukaemias, patients with Down syndrome and acute lymphocytic leukaemia did worse than matched controls. Our results on long-term outcome and the effect of *ETV6-RUNX1* differ from the results of Buitenkamp and colleagues,[2] highlighting the importance of matched cohort analyses.

MRD levels of patients with Down syndrome and acute lymphocytic leukaemia were similar to those of matched controls and more than 90% of the patients with Down syndrome and acute lymphocytic leukaemia had low MRD levels (<0·0001) at the end of consolidation treatment. This finding indicates that the current induction schedules are effective in eradicating leukaemia for patients with Down syndrome and acute lymphocytic leukaemia, similar to matched controls[10]—and are possibly even more effective in patients with Down syndrome and acute lymphocytic leukaemia, considering that some of the included protocols were modified for patients with Down syndrome and acute lymphocytic leukaemia who reduced the treatment intensity during induction (eg, omitting anthracyclines).[3,10] Nevertheless, more patients with Down syndrome and acute lymphocytic leukaemia died during induction than did matched controls, emphasising the need for alternative treatment strategies to reduce early toxicity. Results from the UKALL2003 trial showed that a three-drug induction treatment instead of a four-drug induction treatment in low-risk patients with acute lymphocytic leukaemia (both with and without Down syndrome), resulted in fewer toxic deaths.[13] Patients with Down syndrome and acute lymphocytic leukaemia who had full intensity and lowered intensity induction regimens were included in the present study. However, since the modifications were made only for patients with Down syndrome and acute lymphocytic leukaemia and were often throughout the duration of the protocol, only a few matched patients had induction treatments that were exactly the same. Therefore, we could not address the effect of the different induction regimens on MRD levels or early TRM.

For patients with Down syndrome and acute lymphocytic leukaemia, treatment intensity during maintenance is often modified as part of the standard protocols[10,13] or due to possible dose reduction as a result of excessive toxicity.[4] Despite these modifications, our matched cohort study reports excessive post-induction TRM in patients with Down syndrome and acute lymphocytic leukaemia, both during and after treatment, in addition to an increased number of induction deaths. Our results are partly in line with results from an earlier UKALL2003 study.[27] Scaling down chemotherapy in patients with Down syndrome and acute lymphocytic leukaemia was shown to reduce treatment-related toxicity and mortality without increasing the risk of relapse, although this scenario is probably most relevant to low-risk acute lymphocytic leukaemia populations.[28-30] Simultaneously, as part of a more targeted approach, immunotherapies, like inotuzumab ozogamicin, blinatumomab, or chimeric antigen receptor T-cell therapy, should be studied specifically in individuals with Down syndrome. An ongoing study in North America (NCT03914625) is investigating blinatumomab in combination with chemotherapy for children with newly diagnosed B-cell acute lymphocytic leukaemia, with indivduals with Down syndrome included. Additionally, blinatumomab is an alternative treatment strategy for high-risk patients with Down syndrome and acute lymphocytic leukaemia in the European ALLTogether1 trial (NCT03911128). Unravelling the driving genomic lesions and causes of treatment-related toxicity might point to new, less toxic treatment options for children with Down syndrome and acute lymphocytic leukaemia.

In summary, there is a high need for customised treatment strategies for acute lymphocytic leukaemia in patients with Down syndrome. Novel therapeutic approaches are needed to replace toxic therapy elements with equally effective drugs that have a more favourable safety profile, next to improving supportive care.

Contributors

NM, JMB, CMZ, and MLdB conceptualised this study, analysed the data, and prepared the first draft of the manuscript. AE, RS, MH, SE,

HAdG-K, VHJvdV, and GB provided the patient data for this study. MF supervised the statistical analyses. AV, TT, LD-P, RP, UzS, GE, KS, AVM, and CMZ were the principal investigators involved in this study or were responsible for the patient trials. MLdB was responsible for the Ponte di Lego matched cohort study. NM, JMB, AE, RS, MH, SE, and HAdG-K accessed and verified the raw data. All other authors had access to the data during the study. CMZ made the final descision to submit the manuscript for publication. All authors reviewed the final manuscript.

Declaration of interests

KS reports speaker or advisory board honoraria from Jazz Pharmaceuticals and Servier; speaker fees from Amgen and Medscape; and an educational grant from Servier. CMZ reports grants from Pfizer, Takeda, AbbVie, and Jazz Pharmaceuticals; consulting fees from Novartis, Incyte, Pfizer, Jazz Pharmaceuticals, Takeda, and Abbvie; speaker fees from Pfizer; travel expenses from Jazz Pharmaceuticals; participation on data safety monitoring committees or advisory boards for Novartis, and Incyte; and is co-chair of the Innovative Therapies for Children with Cancer heamatological malignancies committee. GB reports grants from Swedish Society Pediatric Cancer. TT reports foundation funding to Children's Cancer Institute; project funding from Tour de Cure; and ownership of stock or stock options in CSL, Cochlear, Medical Developments International, Osteopore, and Sonic Healthcare. RS reports grants paid to the University of New South Wales from National Health and Medical Research Council Australia, Cancer Counsel New South Wales, and Cancer Australia; and foundation funding to the Children's Cancer Institute from Tour de Cure and Australian Cancer Research Foundation. All other authors declare no competing interests.

Data sharing

Individual participant data are not available to share. Participating study groups should be contacted directly for the original data.

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