Outcomes of paediatric patients with B-cell acute lymphocytic leukaemia with ABL-class fusion in the pre-tyrosine-kinase inhibitor era: a multicentre, retrospective, cohort study

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Summary

Background ABL-class fusion genes other than *BCR–ABL1* have been identified in approximately 3% of children with newly diagnosed acute lymphocytic leukaemia, and studies suggest that leukaemic cells carrying ABL-class fusions can be targeted successfully by tyrosine-kinase inhibitors. We aimed to establish the baseline characteristics and outcomes of paediatric patients with ABL-class fusion B-cell acute lymphocytic leukaemia in the pre-tyrosine-kinase inhibitor era.

Methods This multicentre, retrospective, cohort study included paediatric patients (aged 1–18 years) with newly diagnosed ABL-class fusion (*ABL1* fusion-positive, *ABL2* fusion-positive, *CSF1R* fusion-positive, and *PDGFRB* fusion-positive) B-cell acute lymphocytic leukaemia enrolled in clinical trials of multidrug chemotherapy done between Oct 3, 2000, and Aug 28, 2018, in which tyrosine-kinase inhibitors had not been given as a first-line treatment. Patients from 14 European, North American, and Asia-Pacific study groups of the Ponte di Legno group were included. No patients were excluded, and patients were followed up by individual study groups. Through the Ponte di Legno group, we collected data on the baseline characteristics of patients, including *IKZF1*, *PAX5*, and *CDKN2A/B* deletion status, and whether haematopoietic stem cell transplantation (HSCT) had been done, as well as treatment outcomes, including complete remission, no response, relapse, early death, and treatment-related mortality, response to prednisone, and minimal residual disease (MRD) at end of induction therapy. 5-year event-free survival and 5-year overall survival were estimated by use of Kaplan-Meier methods, and the 5-year cumulative incidence of relapse was calculated by use of a competing risk model.

Findings We identified 122 paediatric patients with newly diagnosed ABL-class fusion B-cell acute lymphocytic leukaemia (77 from European study groups, 25 from North American study groups, and 20 from Asia-Pacific study groups). 64 (52%) of 122 patients were *PDGFRB* fusion-positive, 40 (33%) were *ABL1* fusion-positive, ten (8%) were *CSF1R* fusion-positive, and eight (7%) were *ABL2* fusion-positive. In all 122 patients, 5-year event-free survival was 59·1% (95% CI 50·5–69·1), 5-year overall survival was 76·1% (68·6–84·5), and the 5-year cumulative incidence of relapse was 31·0% (95% CI 22·4–40·1). MRD at the end of induction therapy was high ($\geq 10^{-2}$ cells) in 61 (66%) of 93 patients, and most prevalent in patients with *ABL2* fusions (six [86%] of 7 patients) and *PDGFRB* fusion-positive B-cell acute lymphocytic leukaemia (43 [88%] of 49 patients). MRD at the end of induction therapy of 10^{-2} cells or more was predictive of an unfavourable outcome (hazard ratio of event-free survival in patients with a MRD of $\geq 10^{-2}$ *vs* those with a MRD of $<10^{-2}$ 3·33 [95% CI 1·46–7·56], p=0·0039). Of the 36 (30%) of 119 patients who relapsed, 25 (69%) relapsed within 3 years of diagnosis. The 5-year cumulative incidence of relapse in 41 patients who underwent HSCT (17·8% [95% CI 7·7–31·3]) was lower than in the 43 patients who did not undergo HSCT (45·1% [28·4–60·5], p=0·013), but event-free survival and overall survival did not differ between these two groups.

Interpretation Children with ABL-class fusion B-cell acute lymphocytic leukaemia have poor outcomes when treated with regimens that do not contain a tyrosine-kinase inhibitor, despite the use of high-risk chemotherapy regimens and frequent HSCT upon first remission. Our findings provide a reference for evaluating the potential benefit of first-line tyrosine-kinase inhibitor treatment in patients with ABL-class fusion B-cell acute lymphocytic leukaemia.

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Introduction

Gene expression profiling studies have identified over 20 genetic subtypes of B-cell acute lymphocytic leukaemia in children. Of particular interest is *BCR–ABL1*-like (also known as Philadelphia chromosome-like) B-cell acute lymphocytic leukaemia, because patients with this type have a high risk of relapse. First described in 2009, these patients have a similar gene expression profile as those with *BCR–ABL1* fusion acute lymphocytic leukaemia, even though they are negative for the *BCR–ABL1* fusion.[1,2] *BCR–ABL1*-like B-cell acute lymphocytic leukaemia is characterised by a high frequency of lesions involving ABL-class genes (12–18% of cases), as well as JAK pathway genes (*JAK2* and *EPOR*), and chemokine receptor genes (*CRLF2*) or MEK–ERK pathway genes, or both.[3–5] The ABL-class group of genomic alterations mainly consist of in-frame fusions of tyrosine-kinase- encoding genes, such as *ABL1, ABL2, CSF1R,* and *PDGFRB,* to other genes that are normally expressed during B-cell development. The resulting chimeric proteins have profound tyrosine kinase activity in cells in which the ABL-class genes are usually not abundantly expressed, resulting in the activation of pathways involved in the survival and proliferation of immature lymphoid cells.[6,7]

The addition of tyrosine-kinase inhibitors, such as imatinib and dasatinib, to chemotherapy-based therapies has substantially improved outcomes in children with newly diagnosed *BCR–ABL1* fusion leukaemia.[8–12] Similar to *BCR–ABL1* fusion acute lymphocytic leukaemia, ABL-class fusion acute lymphocytic leukaemia is associated with high-risk features, such as being aged 10 years and older, a high white blood cell count at diagnosis (ie, $>50\times10^9$ cells per L), and high minimal residual disease (MRD) levels at the end of induction therapy (ie, $>10^{-4}$ cells).[5,13] ABL-class fusions are found at a significantly higher frequency in National Cancer Institute (NCI)-defined high-risk patients (4%) than in standard-risk patients (0·2%).[14,15] Imatinib and dasatinib have both shown significant activity in preclinical models of ABL-class fusions, and these findings are consistent with those from *BCR–ABL1* fusion-positive preclinical models.[3,6,7] Given the molecular similarities of ABL-class fusion acute lymphocytic leukaemia to *BCR–ABL1* fusion disease, there is a strong rationale for assessing the potential benefit of tyrosine-kinase inhibitors in these patients. Several anecdotal reports have described excellent responses to additional tyrosine-kinase inhibitor therapy in these patients, but they do not provide information about long-term outcomes.[6,16,17] Two reports published in 2019 highlight the potential activity of tyrosine-kinase inhibitors in patients with ABL-class fusion acute lymphocytic leukaemia, but these reports are limited by small patient numbers and the late introduction of tyrosine-kinase inhibitor therapy.[13,18] The ABL-class fusion cohort therefore remains a heterogeneous group of patients with unverified baseline characteristics and outcomes, particularly when considering those with *ABL1* fusion, *ABL2* fusion, *CSF1R* fusion, and *PDGFRB* fusion-positive acute lymphocytic leukaemia separately.

The Ponte di Legno group consists of more than 20 established acute lymphocytic leukaemia study groups worldwide, and was initiated to investigate outcomes in rare subsets of paediatric patients with newly diagnosed acute lymphocytic leukaemia, for which individual study groups have only a small number of cases. We did a retrospective study to investigate the clinical outcomes of

Research in context

Evidence before this study

In the past decade, it has become clear that ABL-class gene fusions other than *BCR–ABL1* are present in approximately 3% of children with acute lymphocytic leukaemia. Preclinical studies suggest that leukaemic cells carrying ABL-class fusions can be targeted successfully by tyrosine-kinase inhibitors. The addition of tyrosine-kinase inhibitors to the treatment of patients with *BCR–ABL1*-positive acute lymphocytic leukaemia has substantially improved outcomes, but whether treatment with tyrosine-kinase inhibitors improves outcomes in patients with other ABL-class fusions remains unknown. Additionally, the group of patients with ABL-class fusions is heterogeneous and includes patients with *ABL1* fusion, *ABL2* fusion, *CSF1R* fusion, and *PDGFRB* fusion types. The outcomes of patients with these acute lymphocytic leukaemia types is not known because their incidence is low. We did not undertake a systematic search of the literature before this study was done.

Added value of this study

This study was done by the Ponte di Legno group, which consists of more than 20 established acute lymphocytic leukaemia study groups worldwide, and was initiated to investigate outcomes in subsets of paediatric patients with rare, newly diagnosed acute lymphocytic leukaemia. In this study, we investigated the characteristics and outcomes of paediatric patients with ABL-class fusion B-cell acute lymphocytic leukaemia who were included in first-line trials without tyrosine-kinase inhibitors. We found high levels of minimal residual disease after the first course of multidrug chemotherapy and unfavourable survival outcomes in two-thirds of patients, indicating that paediatric patients with ABL-class fusion acute lymphocytic leukaemia responded poorly to multidrug chemotherapy regimens.

Implications of all the available evidence

The results described in this paper will serve as reference to interpret the potential benefit of adding tyrosine-kinase inhibitors to the first-line treatment regimens of paediatric patients with ABL-class fusion B-cell acute lymphocytic leukaemia.

patients with newly diagnosed ABL-class fusion B-cell acute lymphocytic leukaemia enrolled in first-line trials of treatment regimens that did not contain tyrosine-kinase inhibitors. The results described in this study will serve as reference to interpret the potential benefit of adding tyrosine-kinase inhibitors to the first-line treatment regimens of children with ABL-class fusion B-cell acute lymphocytic leukaemia.

Methods

Study design and participants

This multicentre, retrospective, cohort study included paediatric patients (aged 1–18 years) with newly diagnosed ABL-class fusion B-cell acute lymphocytic leukaemia enrolled in clinical trials of multidrug chemotherapy done between Oct 3, 2000, and Aug 28, 2018, in which tyrosine-kinase inhibitors had not been given as a first-line treatment. Patients were collected from 14 national study groups in Europe, North America, and the Asia-Pacific region. No patients were excluded, and patients were followed up by the individual study groups.

The ABL-class fusion B-cell acute lymphocytic leukaemia cohort consisted of patients with *ABL1, ABL2, CSF1R*, and *PDGFRB* fusion. Patients with ABL-class fusion B-cell acute lymphocytic leukaemia were identified by the diagnostic and research laboratories of participating study groups. Patients were retrospectively tested, often for research purposes, to characterise this subset of patients with *BCR–ABL1*-like B-cell acute lymphocytic leukaemia who have a poor prognosis. Patients with B-cell acute lymphocytic leukaemia who were negative for prognostically relevant genetic lesions (ie, *BCR–ABL1, KMT2A*-rearranged, *ETV6–RUNX1, TCF3–PBX1,* and high hyperdiploidy) were subject to total RNA sequencing, RT-PCR analysis, or fluorescence in situ hybridisation analysis, often prompted by cytogenetic or karyotypic evidence for abnormal chromosomal regions affecting 1q25 (*ABL2*), 5q13–34 (*CSF1R* and *PDGFRB*),[19] and 9q34 (*ABL1*), or by the gene expression signatures used to identify patients with *BCR–ABL1*-like B-cell acute lymphocytic leukaemia.[1,3,4,20] Identification of patients with ABL-class fusion B-cell acute lymphocytic leukaemia was dependent on sample availability and the decision to do additional analyses by individual study groups. An example of the methods used by the Dutch Childhood Oncology Group to identify genetic lesions is provided in the appendix (pp 1–2). Some patients with *BCR–ABL1*-like B-cell acute lymphocytic leukaemia included in the study have been presented in publications about new fusion gene discoveries (including *CRLF2*-fusion, *EPOR*-fusion, and *JAK*-fusion),[5,6,19,21] and some patients in a single study group have been included in a publication about outcomes.[13]

In accordance with the Declaration of Helsinki, written informed consent was obtained from the parents or guardians of the children, and the institutional review boards of the contributing study groups approved the use of anonymised patient data for research purposes.

Procedures

Baseline characteristics, including sex, age, white blood cell count at diagnosis, CNS (ie, cerebral spinal fluid containing ≥5 leukocytes per µL and blast cells or signs of CNS involvement, according to the Paediatric Acute Lymphocytic Leukaemia Central Nervous System classification system) or testis involvement, and deletion status of *IKZF1*, *CDKN2A/B*, and *PAX5*, and outcomes, including response to prednisone, complete remission, risk stratification, MRD, haematopoietic stem cell transplantation (HSCT) in first remission, and first event (ie, no response, early death, relapse, second malignancy, and death), of patients were collected from the 14 study groups of the Ponte di Legno group by use of a standard case report form, which was completed by the database representative of the contributing study groups.

Patients were grouped by NCI-defined risk criteria into standard-risk (defined as being aged <10 years and having a white blood cell count of $<50\times10^9$ cells per L) and high-risk groups (aged ≥10 years or a white blood cell count of $\geq50\times10^9$ cells per L, or both, at diagnosis).

Depending on the availability of DNA samples, the presence of *IKZF1* deletions (intragenic or complete deletion), *CDKN2A/B* deletions, and *PAX5* deletions were assessed by use of a multiplex ligation-dependent probe amplification (MLPA) assay (SALSA MLPA kit P335; MRC Holland, Amsterdam, Netherlands). Data on *PAR1* and *ERG* status were not always available in some study groups, therefore, *IKZF1*^{plus} status (ie, *IKZF1* deletions with concomitant *CDKN2A/B*, *PAX5*, or *PAR1* deletions in the absence of *ERG* deletion) could not be assessed as described previously.[22] Instead, we compiled a derivative *IKZF1*^{plus} group of patients who had *IKZF1* deletions with concomitant deletions in *PAX5* or *CDKN2A/B*, or both, which largely (>85%) overlaps with the previously reported definition of *IKZF1*^{plus} status.[22,23]

MRD testing was done by real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements or flow cytometry, depending on the protocol guidelines for each study group. The results of these MRD monitoring methods are comparable according to previously published results, which showed concordance in 97% of cases, and a sensitivity of 0·01% for flow cytometry and 0·001% for PCR.[24] We merged data from both detection methods in this study, defining 1% of monoclear cells as leukaemic cells by flow cytometry as equivalent to 10^{-2} leukaemic cells by PCR analysis of immunoglobulin and T-cell receptor gene rearrangements. In most cases, MRD values were measured at the end of induction therapy, although, less frequently, data were also collected at the end of consolidation. The end of induction therapy was defined as 29–35 days after the start of treatment, and the end of consolidation was defined as 77–80 days after the start of treatment, considering minor differences in timepoints between study protocols.

See Online for appendix

For Berlin-Frankfurt-Münster study group-based trials, data on response to a therapeutic window of 7 days of

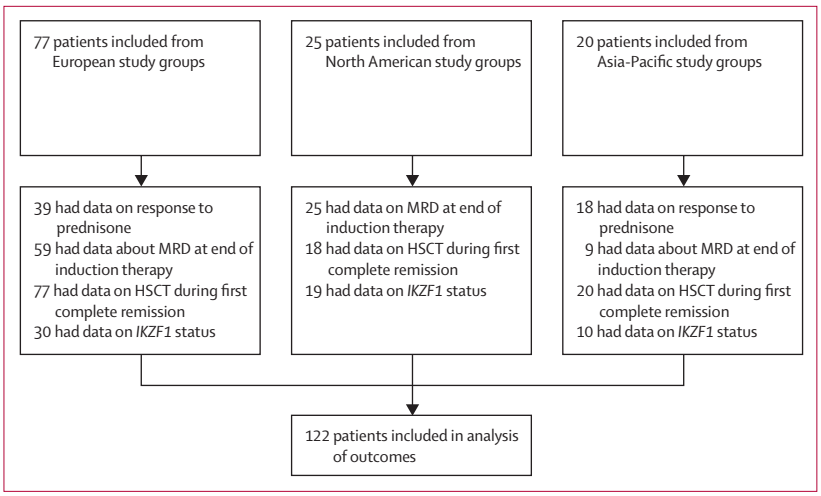


Figure 1: **Study profile**
The distribution of patients with ABL-class fusion B-cell acute lymphocytic leukaemia who had not been exposed to first-line tyrosine-kinase inhibitors by study group is shown. MRD=minimal residual disease. HSCT=haematopoietic stem cell transplantation.

prednisone and one dose of intrathecal methotrexate were collected. A poor prednisone response was defined as the persistence of 1000 or more blasts per µL of peripheral blood on day 8 of treatment, whereas a good response to prednisone was defined as fewer than 1000 blasts per µL of peripheral blood on day 8. Patients received risk-stratified treatment on the basis of the criteria set by each treatment protocol. In this study, patients treated with either standard risk, medium risk, or non-high-risk treatment in individual protocols were collectively assigned to the non-high-risk treatment group, and only patients receiving high-risk therapy were assigned to the high-risk treatment group.

Data on the following treatment outcomes were collected: complete remission, defined as the presence of fewer than 5% leukaemic cells in the bone marrow, recovery of normal haematopoiesis, the absence of peripheral blood leukaemic cells, and no evidence of disease at any other site; early death, defined as death during induction therapy and before complete

	ABL-class fusion-positive (n=122)	*ABL1* fusion-positive (n=40)	*ABL2* fusion-positive (n=8)	*CSF1R* fusion-positive (n=10)	*PDGFRB* fusion-positive (n=64)	p value*
Sex	..	..	..	..	..	0·86†
Male	76 (62%)	25 (63%)	4 (50%)	7 (70%)	40 (63%)	..
Female	46 (38%)	15 (38%)	4 (50%)	3 (30%)	24 (38%)	..
Mean age at diagnosis, years	9·7 (5·1)	7·1 (5·3)	14·0 (4·3)	10·4 (4·5)	10·7 (4·5)	0·0066†
Age group, years						
1–9	54 (44%)	26 (65%)	1 (13%)	3 (30%)	24 (38%)	..
10–18	68 (56%)	14 (35%)	7 (88%)	7 (70%)	40 (63%)	..
Mean white blood cell count at diagnosis, ×10^9 cells per L‡	97·9 (114·8)	99·1 (110·1)	142·4 (71·1)	65·5 (100·4)	96·7 (124·0)	0·079†
White blood cell count group, ×10^9 cells per L						
<50	62/121 (51%)	21/39 (54%)	0	7 (70%)	34 (53%)	..
50 to <100	18/121 (15%)	4/39 (10%)	2 (25%)	1 (10%)	11 (17%)	..
≥100	41/121 (34%)	14/39 (36%)	6 (75%)	2 (20%)	19 (30%)	..
National Cancer Institute-defined risk	..	..	..	..	..	0·081†
Standard risk	28/121 (23%)	14/39 (36%)	0	2 (20%)	12 (19%)	..
High risk	93/121 (77%)	25/39 (64%)	8 (100%)	8 (80%)	52 (81%)	..
CNS involvement	..	..	..	..	..	0·34†
No	112/116 (97%)	38/38 (100%)	8 (100%)	9 (90%)	57/60 (95%)	..
Yes	4/116 (3%)	0	0	1 (10%)	3/60 (5%)	..
Testis involvement	..	..	..	..	..	NA§
No	44/44 (100%)	15/15 (100%)	3/3 (100%)	4/4 (100%)	22/22 (100%)	..
Yes	0	0	0	0	0	..
IKZF1 status	..	..	..	..	..	0·44†
Wild-type *IKZF1*	23/59 (39%)	4/16 (25%)	2/4 (50%)	2/7 (29%)	15/32 (47%)	..
Deletion of *IKZF1*	36/59 (61%)	12/16 (75%)	2/4 (50%)	5/7 (71%)	17/32 (53%)	..
IKFZ1, PAX5, and CDKN2A/2B status	..	..	..	..	..	0·72
Deletion of *IKZF1* only	13/59 (22%)	5/12 (42%)	0	2/5 (40%)	6/17 (35%)	..
Deletion of *IKZF1* and *PAX5* or *CDKN2A/B*, or *IKZF1*, *PAX5*, and *CDKN2A/B* deletion	23/59 (39%)	7/12 (58%)	2/2 (100%)	3/5 (60%)	11/17 (65%)	..

Data are n (%), mean (SD), or n/N (%). NA=not applicable. *Calculated by Pearson's χ^2 test. †Estimated p value because the number of patients was fewer than five for some variables. ‡Excludes one patient with missing data. §p value not calculated because there were no patients with involvement of the testis.

Table 1: **Characteristics and risk factors of patients**

	ABL-class fusion-positive (n=122)	*ABL1* fusion-positive (n=40)	*ABL2* fusion-positive (n=8)	*CSF1R* fusion-positive (n=10)	*PDGFRB* fusion-positive (n=64)	p value*
Response to prednisone	..	..	..	..	..	0·0002†
Good response	29/57 (51%)	13/14 (93%)	2/5 (40%)	4/4 (100%)	10/34 (29%)	..
Poor response	28/57 (49%)	1/14 (7%)	3/5 (60%)	0	24/34 (71%)	..
Treatment group	..	..	..	..	..	0·0023†
Non-high risk	28/121 (23%)	16/39 (41%)	1 (13%)	4 (40%)	7 (11%)	..
High risk	93/121 (77%)	23/39 (59%)	7 (88%)	6 (60%)	57 (89%)	..
MRD at the end of induction therapy	..	..	..	..	..	<0·0001†
Negative	5/93 (5%)	3/29 (10%)	0	1/8 (13%)	1/49 (2%)	..
$<10^{-4}$‡	11/93 (12%)	8/29 (28%)	0	3/8 (38%)	0	..
10^{-4} to $<10^{-2}$	16/93 (17%)	9/29 (31%)	1/7 (14%)	1/8 (13%)	5/49 (10%)	..
$\geq 10^{-2}$ cells	61/93 (66%)	9/29 (31%)	6/7 (86%)	3/8 (38%)	43/49 (88%)	..
Events	..	..	..	..	..	NA§
No event (complete remission)	72 (59%)	27 (68%)	3 (38%)	8 (80%)	34 (53%)	..
Any event	50 (41%)	13 (33%)	5 (63%)	2 (20%)	30 (47%)	..
Type of event						
Early death	3 (2%)	1 (3%)	0	0	2 (3%)	..
No response	3 (2%)	0	0	0	3 (5%)	..
Relapse	35 (29%)	12 (30%)	2 (25%)	2 (20%)	19 (30%)	..
Second malignancy	2 (2%)	0	0	0	2 (3%)	..
Death during first remission	7 (6%)	0	3 (38%)	0	4 (6%)	..
High-risk treated patients who did not undergo HSCT¶||	43/84 (51%)	13/43 (30%)	1/43 (2%)	4/43 (9%)	25/43 (58%)	..
Complete remission	25/43 (58%)	8/13 (62%)	0	3/4 (75%)	14/25 (56%)	..
Relapse	18/43 (42%)	5/13 (38%)	1/1 (100%)	1/4 (25%)	11/25 (44%)	..
High-risk treated patients who underwent HSCT¶	41/84 (49%)	8/41 (20%)	2/41 (5%)	2/41 (5%)	29/41 (71%)	..
Second complete remission	25/41 (61%)	4/12 (33%)	1/3 (33%)	1/3 (33%)	19/48 (40%)	..
Events after HSCT§						
Any event	16/41 (39%)	4/8 (50%)	1/2 (50%)	1/2 (50%)	10/20 (50%)	..
Relapse	7/41 (17%)	1/8 (13%)	1/2 (50%)	1/2 (50%)	4/20 (20%)	..
Second malignancy	2/41 (5%)	0	0	0	2/20 (10%)	..
Treatment-related mortality	7/41 (17%)	3/8 (38%)	0	0	4/20 (20%)	..

Data are n/N (%) or n (%). MRD=minimal residual disease. NA=not applicable. HSCT=haematopoietic stem cell transplantation. *Calculated by Pearson χ^2 test. †Estimated p value because the number of patients was fewer than five for some variables. ‡Includes patients with positive but not quantifiable MRD. §p value not calculated because of time-related occurrence of events, which were compared in the survival analyses. ¶Three patients who had no response to treatment (all *PDGFRB* fusion-positive) also received HSCT; one relapsed, one had treatment-related mortality, and one achieved second complete remission. These three patients were included in the HSCT outcome analysis. ||A waiting time to HSCT of 6 months was taken as the landmark, and outcome events were only considered if they occurred after this landmark.

Table 2: **Risk stratification and response of patients with ABL-class fusion-positive B-cell acute lymphocytic leukaemia**

remission; treatment-related mortality, defined as any death during first complete remission (ie, time from diagnosis); no response, defined as not achieving complete remission after two courses of chemotherapy; and relapse, defined as disease recurrence after initially achieving complete remission. The time between diagnosis and the start of treatment was typically 0–2 days.

Outcomes

The outcomes were 5-year event-free survival (defined as time from diagnosis to first event, which included no response, early death, relapse, second malignancy, and death during first remission), 5-year overall survival (defined as time from diagnosis to date of death from any cause), and the 5-year cumulative incidence of relapse and treatment-related mortality, analysed in all patients included in the study. Individuals without an event were censored at the last date of contact. We also measured 5-year event-free survival in patients who had information on MRD at end of induction therapy, response to prednisone, *IKZF1* deletion, and the need for high-risk treatment or HSCT.

Statistical analysis

The Pearson's χ^2 and Kruskal-Wallis tests were used to compare age, white blood cell counts, and MRD levels between *ABL1* fusion, *ABL2* fusion, *CSF1R* fusion, and *PDGFRB* fusion-positive patients. The Wilcoxon matched-pairs test was used to compare MRD at end of

induction and end of consolidation treatment for patients with available data for both timepoints. A competing risk model considering relapse and death was used to estimate the cumulative incidence of relapse and the cumulative incidence of treatment-related mortality from first diagnosis in patients who achieved complete remission. The Gray test was used to compare the cumulative incidence of relapse between patients. The Kaplan-Meier method was used to estimate event-free survival and overall survival. Kaplan-Meier curves of event-free survival and overall survival between ABL-class fusion groups were compared by use of the log-rank test, and the 5-year survival percentages and 95% CIs are presented. To quantify the effect of risk factors on event-free survival, including multivariate analyses, a Cox proportional hazard regression model was used to estimate the hazard ratios (HRs) and the 95% CIs. The effect of HSCT was investigated by use of a landmark approach to avoid immortal time bias. A waiting time to HSCT of 6 months was taken as the landmark time period, and outcome events were only considered if they occurred after the landmark. Statistical analyses were done by use of SPSS (version 25). All analyses involving the competing risks model were done by use of the cmprsk package (version 2·2-7) in R (version 3.2.2). All Kaplan-Meier plots for event-free survival, overall survival, and cumulative incidence of relapse start at time of diagnosis.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all of the data and the final responsibility to submit for publication.

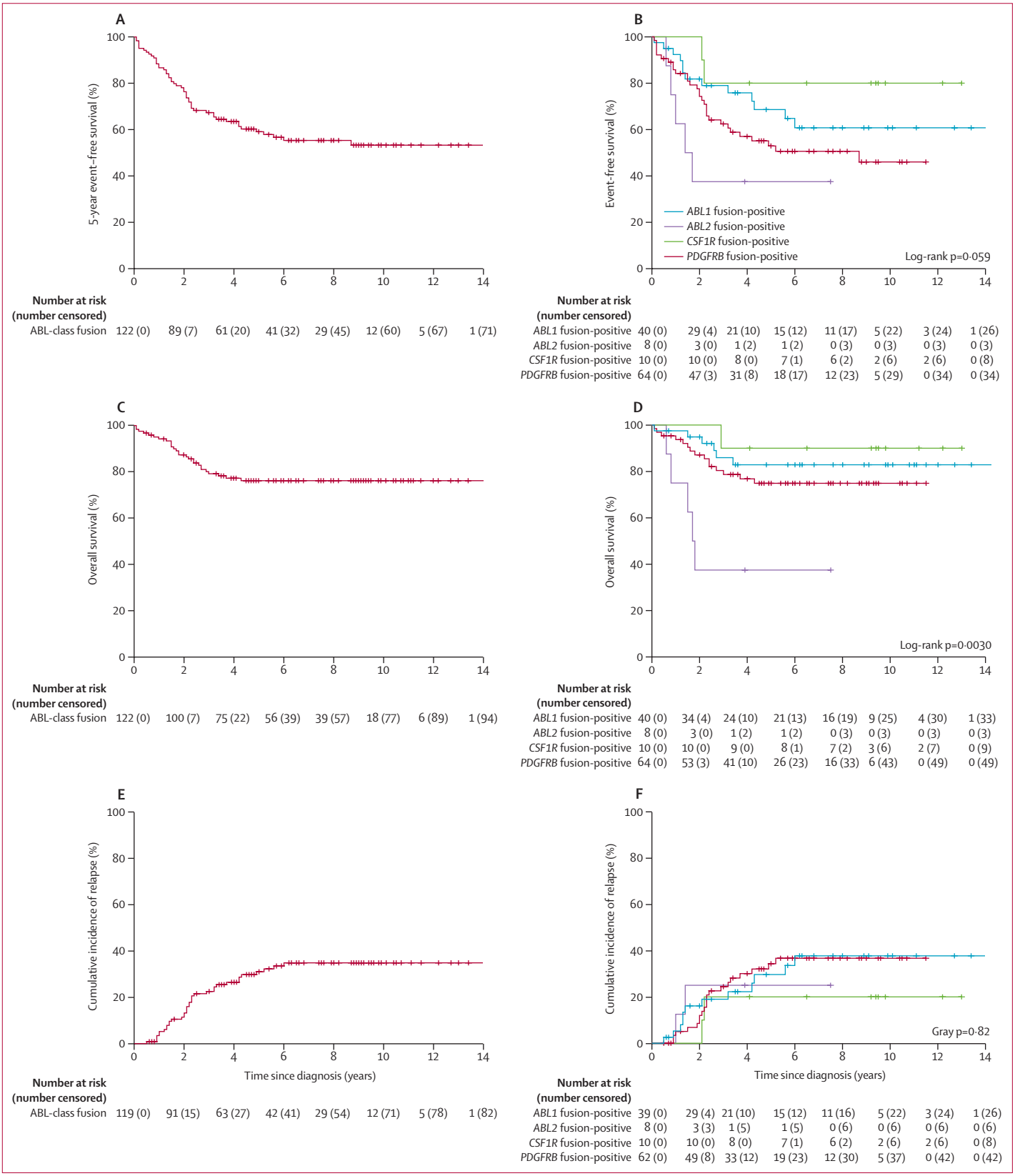
Results

We identified 122 paediatric patients with newly diagnosed ABL-class fusion B-cell acute lymphocytic leukaemia, who had not received tyrosine-kinase inhibitor treatment as a first-line therapy (77 from European study groups, 25 from North American study groups, and 20 from Asia-Pacific study groups; figure 1). The number of patients enrolled by study group, treatment protocol, and NCI-defined risk group is provided in the appendix (p 3). Data for 33 patients from the AIEOP-BFM study group were included in a previous single-treatment protocol study.[13] 84 (69%) of 122 patients were diagnosed between Oct 3, 2000, and Nov 20, 2009, and the remaining 38 (31%) patients were diagnosed between Feb 22, 2000, and Aug 28, 2018. Since most study groups selectively screened for ABL-class fusions, the frequency of ABL-class fusion types (appendix p 6) might not represent the population-based distribution of these types. Most ABL-class fusions involved *PDGFRB* (64 [52%] of 122 patients) and *ABL1* (40 [33%] patients), with only a few patients who had fusions involving *CSF1R* (ten [8%] patients) and *ABL2* (eight [7%] patients). 14 different fusion partner genes were found, and their frequencies according to ABL-class fusion type are outlined in the appendix (pp 4, 6). *EBF1* was the predominant partner for *PDGFRB* (50 [78%] of 64 patients), *ZMIZ1* for *ABL1* (16 [40%] of 40 patients), *SSBP2* for *CSF1R* (eight [80%] of ten patients), and *RCSD1* and *ZH3HAV1* for *ABL2* (four [50%] of eight patients for each).

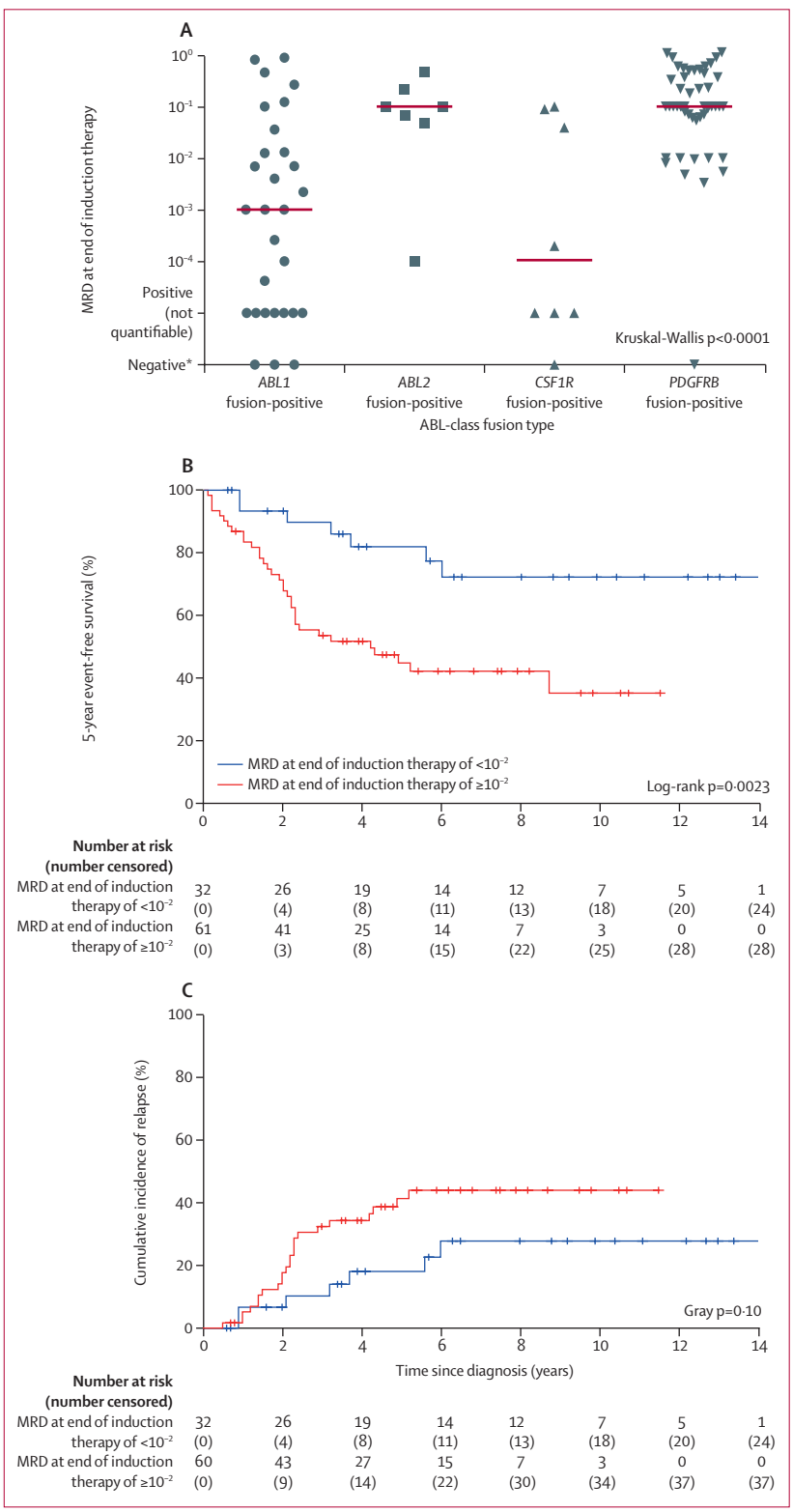
Patient characteristics are shown in table 1. ABL-class fusions were most commonly observed in patients classified as high risk according to NCI criteria (93 [77%] of 121 patients), and were skewed towards high risk for all four ABL-class fusion types (table 1). Age and white blood cell counts at diagnosis varied significantly between the four ABL-class fusion types, with the highest mean age and white blood cell counts in patients with *ABL2*-fusion positive B-cell acute lymphocytic leukaemia (table 1; appendix p 7). CNS involvement at diagnosis was identified in four (3%) of 116 patients in whom these data were available, including one patient with *CSF1R* fusion-positive and three patients with *PDGFRB* fusion-positive B-cell acute lymphocytic leukaemia. No testicular involvement was observed in the 44 male patients with available data. The male-to-female ratio of patients was approximately 2:1, and did not vary between ABL-class fusion types (table 1). 36 (61%) of 59 patients tested for the deletion status of *IKZF1*, *PAX5*, and *CDKN2A/B* had a deletion in *IKZF1*. A deletion in *IKZF1* only was identified in 13 (22%) of 59 patients, and 23 (39%)patients had a deletion in *IKZF1* and *PAX5* or *CDKN2A/B*, or both. The frequency of an *IKZF1* deletion was not significantly different among the ABL-class fusion types (table 1). Neither an *IKZF1* deletion nor the *IKZF1*^{plus} genotype was associated with an unfavourable outcome when compared with patients with wild-type *IKZF1* (appendix p 8).

Response to prednisone as a single systemic agent was assessed in 57 patients, of whom 28 (49%) had a poor response, with the frequency of a poor response varying significantly among the different ABL-class fusion types (table 2). A poor prednisone response was not predictive of an unfavourable clinical outcome (5-year event-free survival HR 1·46 [95% CI 0·65–3·29], p=0·35; appendix p 9). Non-high-risk treatment was given to 28 (23%) of 121 patients and high-risk treatment was given to 93 (77%) of patients, according to individual treatment protocols (table 2). Early death occurred in three (2%) of 122 patients, and seven (6%) patients died during complete remission. Three (2%) patients were non-responders at the end of consolidation (table 2). These patients continued on their protocol-assigned treatment and

Figure 2: **Outcomes**
Event-free survival of 122 patients (A), and of these patients grouped by ABL-class fusion type (B). Overall survival of 122 patients (C), and of these patients grouped by ABL-class fusion type (D). Cumulative incidence of disease relapse in 119 patients (E), and in these patients grouped by ABL-class fusion type (F). Three patients were excluded because of early death.


A
5-year event-free survival (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Number at risk (number censored)
ABL-class fusion 122 (0) 89 (7) 61 (20) 41 (32) 29 (45) 12 (60) 5 (67) 1 (71)
B
Event-free survival (%)
ABL1 fusion-positive
ABL2 fusion-positive
CSF1R fusion-positive
PDGFRB fusion-positive
Log-rank p=0·059
Number at risk (number censored)
ABL1 fusion-positive 40 (0) 29 (4) 21 (10) 15 (12) 11 (17) 5 (22) 3 (24) 1 (26)
ABL2 fusion-positive 8 (0) 3 (0) 1 (2) 1 (2) 0 (3) 0 (3) 0 (3) 0 (3)
CSF1R fusion-positive 10 (0) 10 (0) 8 (0) 7 (1) 6 (2) 2 (6) 2 (6) 0 (8)
PDGFRB fusion-positive 64 (0) 47 (3) 31 (8) 18 (17) 12 (23) 5 (29) 0 (34) 0 (34)
C
Overall survival (%)
Number at risk (number censored)
ABL-class fusion 122 (0) 100 (7) 75 (22) 56 (39) 39 (57) 18 (77) 6 (89) 1 (94)
D
Overall survival (%)
Log-rank p=0·0030
Number at risk (number censored)
ABL1 fusion-positive 40 (0) 34 (4) 24 (10) 21 (13) 16 (19) 9 (25) 4 (30) 1 (33)
ABL2 fusion-positive 8 (0) 3 (0) 1 (2) 1 (2) 0 (3) 0 (3) 0 (3) 0 (3)
CSF1R fusion-positive 10 (0) 10 (0) 9 (0) 8 (1) 7 (2) 3 (6) 2 (7) 0 (9)
PDGFRB fusion-positive 64 (0) 53 (3) 41 (10) 26 (23) 16 (33) 6 (43) 0 (49) 0 (49)
E
Cumulative incidence of relapse (%)
Time since diagnosis (years)
Number at risk (number censored)
ABL-class fusion 119 (0) 91 (15) 63 (27) 42 (41) 29 (54) 12 (71) 5 (78) 1 (82)
F
Cumulative incidence of relapse (%)
Gray p=0·82
Time since diagnosis (years)
Number at risk (number censored)
ABL1 fusion-positive 39 (0) 29 (4) 21 (10) 15 (12) 11 (16) 5 (22) 3 (24) 1 (26)
ABL2 fusion-positive 8 (0) 3 (3) 1 (5) 1 (5) 0 (6) 0 (6) 0 (6) 0 (6)
CSF1R fusion-positive 10 (0) 10 (0) 8 (0) 7 (1) 6 (2) 2 (6) 2 (6) 0 (8)
PDGFRB fusion-positive 62 (0) 49 (8) 33 (12) 19 (23) 12 (30) 5 (37) 0 (42) 0 (42)

reached complete remission; one patient remained in complete remission, one relapsed, and one patient died of HSCT-related toxicity.



5-year event-free survival in the whole cohort was 59·1% (95% CI 50·5–69·1; figure 2A), with median follow-up of 6·7 years (IQR 3·9–9·4) in those without an event. 5-year event-free survival was 37·5% (95% CI 15·3–91·7) in patients who were *ABL2* fusion-positive, 52·9% (41·5–67·5) in those who were *PDGFRB* fusion-positive, 68·6% (54·5–86·3) in those who were *ABL1* fusion-positive, and 80·0% (58·7–100·0) in those who were *CSF1R* fusion-positive, and did not differ significantly between the groups (p=0·059; figure 2B).

There was no significant difference in the 5-year event-free survival between non-high-risk treated patients (70·7% [95% CI 54·3–92·1]) and high-risk treated patients (55·4% [45·9–67·1], p=0·22; appendix p 10). Most patients (52 [81%] of 64) with *PDGFRB* fusion-positive B-cell acute lymphocytic leukaemia were classified as high risk according to NCI criteria and had high MRD levels at the end of induction therapy (table 2). The few patients with *PDGFRB* fusion-positive B-cell acute lymphocytic leukaemia who received non-high-risk treatment had a poor outcome, with six (86%) of seven non-high-risk treated patients who had an event (5-year event-free survival HR 28·6% [8·9–92·2]) compared with 24 (42%) of 57 high-risk treated patients (56·5% [44·5–71·8], p=0·032; appendix p 10).

5-year overall survival in the whole cohort was 76·1% (95% CI 68·6–84·5; figure 2C). Overall survival was significantly different between the groups (p=0·0030): 37·5% (15·3–91·7) in patients who were *ABL2* fusion-positive, 74·8% (64·6–86·8) in those who were *PDGFRB* fusion-positive, 82·9% (71·2–96·4) in those who were *ABL1* fusion-positive, and 90·0% (73·2–100·0) in those who were *CSF1R* fusion-positive (figure 2D).

The 5-year cumulative incidence of relapse in the whole cohort was 31·0% (95% CI 22·4–40·1; figure 2E). Of the 36 (30%) of 119 patients who relapsed, 25 (69%) relapsed within 3 years of diagnosis. Most relapses occurred in the bone marrow (29 [83%] of 35 patients; relapse location was not reported for one patient). Extramedullary relapse (mostly in the CNS) was observed either alone or combined with medullary relapse in 11 (31%) of 36 patients who relapsed. 20 (56%) of 36 patients who relapsed remained alive during the second complete remission. Outcomes varied among the different ABL-class fusion types. The 5-year cumulative incidence of relapse was 25·0% (2·7–58·7) in patients who were *ABL2* fusion-positive, 29·6% (15·0–45·9) in those who were *ABL1* fusion-positive, 34·3% (22·0–47·0) in those

Figure 3: **MRD at the end of induction therapy**
(A) Absolute MRD at the end of induction therapy according to ABL-class fusion type. Red lines indicate median absolute minimal residual disease values. (B) Kaplan-Meier plot showing the 5-year event-free survival of patients according to the degree of MRD at the end of induction therapy. (C) Kaplan-Meier plot showing cumulative incidence of disease relapse according to the degree of MRD at the end of induction therapy. MRD=minimal residual disease. *Beyond the limit of detection.

who were *PDGFRB* fusion-positive, and 20·0% (2·6–49·0) in those who were *CSF1R* fusion-positive (figure 2F; p=0·82).

93 (76%) of 122 patients had information on MRD at the end of induction therapy. MRD was detectable at the end of induction therapy in 88 (95%) of 93 patients and was significantly different among the four ABL-class fusion types (p<0·0001; table 2, figure 3A). Patients with *ABL1* fusion-positive B-cell acute lymphocytic leukaemia had the lowest levels of MRD at the end of induction therapy; 20 (69%) of 29 patients had less than 10^{-2}, although only 11 (38%) patients had less than 10^{-4} (table 2). Patients who were *ABL1* fusion-positive had significantly lower levels of MRD at the end of induction therapy than those who were *ABL2* fusion-positive (p=0·044) and *PDGFRB* fusion-positive (p=0·041). Similarly, patients who were *CSF1R* fusion-positive had significantly lower levels of MRD at the end of induction therapy than those who were *ABL2* fusion-positive (p<0·0001) and *PDGFRB* fusion-positive (p=0·0010). Levels of MRD did not differ significantly across the 14 ABL-class fusion partner genes (appendix p 11). Of the 41 patients with available data on MRD at both the end of induction therapy and at the end of consolidation, seven (17%) had negative or non-quantifiable MRD at the end of induction therapy and 16 (39%) had negative or non-quantifiable disease at the end of consolidation. A paired test indicated that MRD levels were reduced at the end of consolidation therapy compared with at the end of induction therapy in patients for whom both sets of data were available (p<0·0001; appendix p 12). Because of the refractory nature of ABL-class fusion acute lymphocytic leukaemia, we used a MRD threshold of 10^{-2} to compare outcomes. Patients with MRD levels at the end of induction therapy of 10^{-2} or more had an unfavourable 5-year event-free survival of 44·7% (95% CI 33·2–60·4) compared with 81·9% (68·7–97·7) in those with MRD levels of less than 10^{-2} (HR 3·34 [95% CI 1·47–7·60], p=0·0023; figure 3B). In multivariate Cox regression models, the prognostic value of MRD for event-free survival appeared to be independent of NCI-defined risk group and treatment group (appendix p 5). The 5-year cumulative incidence of relapse in patients with MRD levels at the end of induction therapy of less than 10^{-2} cells was 18·1% (95% CI 6·4–34·6) and was 41·1% (27·7–54·5) in those with levels of 10^{-2} cells or more (p=0·10; figure 3C).

Of 115 patients for whom HSCT status was reported, two (2%) non-high-risk treated patients and 41 (36%) high-risk treated patients received a HSCT during the first complete remission. The median time to transplantation was 6·7 months (IQR 5·8–7·9). The clinical outcomes of the 41 high-risk treated patients who received HSCT were compared with the 43 high-risk treated patients who did not receive HSCT and had survived for at least 6 months from diagnosis without any event (landmark analysis). Sixteen events occurred after transplantation (seven relapses, seven deaths during the second complete remission, and two second malignancies), compared with 18 events (all relapses) in those who did not receive a transplantation. The HSCT-related mortality was high (seven [17%] of 41 patients), and six (86%) of the seven HSCT-related deaths occurred in patients who had the procedure before 2010. The relapses and deaths during second complete remission (indicative of HSCT-related or treatment-related mortality) mainly occurred within the first 2 years after transplantation, after which point the cumulative incidence of relapse and event-free survival curves stabilised (appendix p 13). By contrast, relapses occurred up to 5 years after the landmark in the group of patients who did not receive HSCT (appendix p 13). The estimated 5-year cumulative incidence of relapse differed significantly between patients who received HSCT (17·8% [95% CI 7·7–31·3]) and those who did not (45·1% [28·4–60·5], p=0·013), but 5-year event-free survival and 5-year overall survival estimates did not differ significantly between these two groups (appendix p 13).

Discussion

This study by the Ponte di Legno group shows that the patients with ABL-class fusion B-cell acute lymphocytic leukaemia, particularly those with *ABL2* fusion-positive and *PDGFRB* fusion-positive types, are characterised by high-risk features, a high frequency of poor prednisone response, high levels of MRD at the end of induction therapy, and unfavourable long-term outcomes. The results showed that, in this cohort of patients who had not received tyrosine-kinase inhibitors, 5-year event-free survival was 59·1% (95% CI 50·5–69·1), 5-year overall survival was 76·1% (68·6–84·5), and 5-year cumulative incidence of relapse was 31·0% (22·4–40·1). These outcomes are inferior to those of other paediatric patients with newly diagnosed B-cell acute lymphocytic leukaemia who have received contemporary treatment protocols (ie, a 5-year event-free survival of >85%, a 5-year overall survival of >90%, and a 5-year cumulative incidence of relapse of <8%).[25,26] Our study also shows that outcomes vary among the four different ABL-class fusion types, in which patients with *ABL2* fusion-positive and *PDFGRB* fusion-positive B-cell acute lymphocytic leukaemia have the most unfavourable baseline characteristics of an older age and a high white blood cell count, and high levels of MRD at the end of induction therapy.

In general, patients with ABL-class fusion B-cell acute lymphocytic leukaemia are characterised by an older age and a high white blood cell count at diagnosis, and 75% are classified as high risk according to NCI criteria compared with 30–35% of the general population of paediatric patients with acute lymphocytic leukaemia.[26,27] The proportion of patients with ABL-class fusion B-cell acute lymphocytic leukaemia who have CNS involvement is low (3%), but similar to that observed in NCI-defined high-risk patients in a large reference cohort (2·4%).[28] By contrast, a poor prednisone response is more frequent in patients with ABL-class fusion B-cell acute lymphocytic

leukaemia (49%) than in reference cohorts of patients with newly diagnosed acute lymphocytic leukaemia (<10%).[25,26] 66% of patients with ABL-class fusions have a high and prognostically unfavourable level of MRD at the end of induction therapy of 10^{-2} cells or more compared with less than 10% of patients with newly diagnosed acute lymphocytic leukaemia in reference cohorts.[25,26] In our study, only 16 (39%) of 41 patients had negative or non-quantifiable MRD levels at the end of consolidation therapy, which is much lower than in other subsets of patients with B-cell acute lymphocytic leukaemia (eg, 2470 [78%] of 3176 newly diagnosed patients with B-cell acute lymphocytic leukaemia in the AIEOP-BFM ALL 2000 study).[26] Positive MRD at this late timepoint is associated with a high risk of relapse and is often used as an indication for HSCT. Considering the strong prognostic role of MRD, reducing MRD levels in patients with ABL-class fusion B-cell acute lymphocytic leukaemia during the initial months of therapy is an important target for decreasing the risk of relapse and for reducing the intensity of treatment (eg, by avoiding HSCT) and the associated complications and mortality.

In our study, the frequency of *IKZF1* deletions in patients was high (36 [61%] of 59 patients) and similar to the frequency of *IKZF1* deletions observed in patients with *BCR–ABL1* fusion acute lymphocytic leukaemia (75%), both of which are markedly higher than the 15% of paediatric patients with *BCR–ABL1*-negative disease.[2,4,29] We found that *IKZF1* deletions, with or without additional deletions in *PAX5* or *CDKN2A/B*, or both, did not have added prognostic value compared with *IKZF1* wild-type ABL-class fusion acute lymphocytic leukaemia, which contrasts with findings in patients with non-ABL-class fusion B-cell acute lymphocytic leukaemia.[22] Therefore, treatment should not be modified on the basis of the presence of an *IKZF1* deletion in patients with ABL-class fusion B-cell acute lymphocytic leukaemia.

Even though it is possible that patients with a high risk of relapse were selected to undergo HSCT during the first complete remission, we found that the overall survival of patients in our cohort was similar between those who received chemotherapy alone or HSCT during the first complete remission. The reduced number of relapses following HSCT was counterbalanced by the number of treatment-related deaths. Similar results were observed in a 2019 study of AIEOP-BFM trials, which compared patients with ABL-class fusion B-cell acute lymphocytic leukaemia, including those who received tyrosine-kinase inhibitors and those who did not, treated with chemotherapy alone or HSCT.[13] Considering a 6-month waiting time for HSCT, in a landmark analysis, we found that relapses occurred over a longer time frame of 5·5 years from diagnosis in the group of patients who did not receive a transplantation compared with the early relapses (ie, within 2·5 years of diagnosis) observed in the group who did receive a transplantation. The occurrence of early relapse after HSCT is a known observation in acute lymphocytic leukaemia, and indicates the failure of the conditioning regimen and the intended immune control by the engrafted immune cells. In our study, the frequency of early relapse in patients who had received HSCT was not associated with a specific type of ABL-class fusion.

Data collected on patients included in our cohort were limited to the first-line treatment up to the occurrence of the first event. The 122 patients were given more than 20 different treatment protocols (1–22 patients in each protocol) between 2000 and 2018, which did not allow separate outcome analyses for each protocol. MRD-guided risk stratification started to be used from 2000 onwards but was not implemented in all protocols in the same way. Even though data on MRD at the end of induction therapy were available for 93 (76%) of 122 patients, only 41 (34%) of 122 patients also had MRD evaluated at the end of consolidation. Over the study period (2000–18), an incremental improvement in the overall survival of paediatric patients with B-cell acute lymphocytic leukaemia was observed; for instance, overall survival in paediatric patients in the Netherlands increased from 86% in 2000–04, to 91% in 2005–09, and to 93% in 2010–15.[30] 84 (69%) of 122 patients with ABL-class fusion B-cell acute lymphocytic leukaemia in our study were diagnosed before 2010, suggesting that there was a decline in patients recruited after this time, possibly because of the increased use of tyrosine-kinase inhibitors as a first-line therapy in recent years. The HSCT-related mortality was high, and six (86%) of seven transplantation-related deaths occurred in patients who received the transplantation before 2010. Further analysis of possible reasons for HSCT failure was limited by the fact that details of the procedure, including conditioning, donor type, stem cell source, and levels of MRD before HSCT, were not available. Our study was not designed to evaluate the effect of HSCT in patients with ABL-class fusion B-cell acute lymphocytic leukaemia, and the number of patients was too small to draw conclusions on the effectiveness of HSCT as an effective consolidation therapy during first complete remission. Similarly, no data were collected on the use of tyrosine-kinase inhibitors or immunotherapies as second-line treatments in our study. Given the study period, it is unlikely that patients included in our analysis were given immunotherapies as a first-line treatment as part of the trial protocols.

The signalling pathways activated by ABL-class fusions strongly suggest that patients with this type of acute lymphocytic leukaemia might benefit from the addition of tyrosine-kinase inhibitors to combination chemotherapy. Preclinical in vitro (in Ba/F3 and $Arf^{-/-}$ cell lines), ex vivo (in patient cells), and in vivo (mouse models) studies, all provide evidence that leukaemic cells harbouring ABL-class fusions are sensitive to different tyrosine-kinase inhibitors, including first-generation (imatinib), second-generation (eg, dasatinib, bosutinib), and third-generation (eg, ponatinib) tyrosine-kinase inhibitors.[3,6,7] Tyrosine-

kinase inhibitors were also efficacious in several case studies of children with largely refractory or relapsed acute lymphocytic leukaemia (appendix pp 14–15).[13,16–18] Furthermore, a 2019 study done in France reported that all eight paediatric patients with ABL-class fusion acute lymphocytic leukaemia, positive for MRD at the end of induction therapy, received tyrosine-kinase inhibitor treatment and had achieved and remained in first complete remission for 11–62 months' follow-up.[18] Similarly, the AIEOP-BFM group reported in 2019 that tyrosine-kinase inhibitors administered at different stages of therapy resulted in only one relapse among 13 children with ABL-class fusion leukaemia.[13] Together, these studies show that tyrosine-kinase inhibitors can be beneficial to patients with ABL-class fusion acute lymphocytic leukaemia. However, evidence to show that tyrosine-kinase inhibitors are effective in patients with the *CSF1R* fusion is missing and limited to preclinical studies showing some sensitivity of *CSF1R* fusion-positive cells to tyrosine-kinase inhibitors.[8,31]

Targeting ABL-class fusion lesions with tyrosine-kinase inhibitors could be as effective as their use in children with *BCR–ABL1*-positive acute lymphocytic leukaemia.[8,9,11,32] The most promising results have been observed by giving tyrosine-kinase inhibitors continuously over 1–2 years to patients with *BCR–ABL1*-positive acute lymphocytic leukaemia.[9,11] Given that the 5-year event-free survival of patients (including a high proportion of those who underwent allogeneic HSCT) included in our study was 59%, the addition of tyrosine-kinase inhibitors to first-line therapies could improve long-term outcomes in patients with ABL-class fusion B-cell acute lymphocytic leukaemia.

In conclusion, this Ponte di Legno study shows that without tyrosine-kinase inhibitors, the outcome for children with ABL-class fusion B-cell acute lymphocytic leukaemia is highly unfavourable. The availability of tyrosine-kinase inhibitors that have been shown to be safe and active when combined with chemotherapy in patients with *BCR–ABL1* positive B-cell acute lymphocytic leukaemia will expedite the use of tyrosine-kinase inhibitor-containing combination therapies in patients with ABL-class fusion B-cell acute lymphocytic leukaemia. This study establishes the outcome standard to which these tyrosine-kinase inhibitor-containing therapies can be compared.

Contributors

The project was conceptualised by RP and MLdB. Patient characteristics and clinical outcome data were collected by the participating study groups and were provided via the chairs of each study group (MS, AV, GE, TI, AY, LD-P, NK, CGM, MLL, AA, MZ, SE, and AB). Data were collected centrally and curated by HAdG-K and MLdB. Statistical analyses and computing in R were done by HAdG-K, MF, and JMB. Data were interpreted by MLdB, GuC, MLL, AVM, and RP. The manuscript was written by MLdB and revised by all coauthors. The final version of the manuscript was approved by all coauthors.

Declaration of interests

GuC reports personal fees from Jazz Pharmaceuticals and Novartis outside the submitted work. SPH reports personal fees from Novartis and Amgen; and financial support from Amgen, outside the submitted work. CGM reports personal fees from Illumina, and grants from Loxo Oncology, during the conduct of the study; and grants from AbbVie and Pfizer, and personal fees from Amgen, outside the submitted work. MS reports grants from SHIRE, Jazz Pharmaceuticals, Servier, SigmaTau, Amgen, and Novartis during the conduct of the study; and personal fees from SHIRE, Servier, and JazzPharma outside the submitted work. All other authors declare no competing interests.

Data sharing

Requests to receive de-identified study data can be submitted to the corresponding author, and should include a description of the research question.

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