

LYMPHOID NEOPLASIA

Independent prognostic value of *BCR-ABL1*-like signature and *IKZF1* deletion, but not high *CRLF2* expression, in children with B-cell precursor ALL

Arian van der Veer,¹ Esmé Waanders,^{2,3} Rob Pieters,^{1,4} Marieke E. Willemse,¹ Simon V. Van Reijmersdal,^{2,3} Lisa J. Russell,⁵ Christine J. Harrison,⁵ William E. Evans,⁶ Vincent H. J. van der Velden,⁷ Peter M. Hoogerbrugge,⁸ Frank Van Leeuwen,⁸ Gabriele Escherich,⁹ Martin A. Horstmann,⁹ Leila Mohammadi Khankahdani,¹ Dimitris Rizopoulos,¹⁰ Hester A. De Groot-Kruseman,⁴ Edwin Sonneveld,⁴ Roland P. Kuiper,^{2,3} and Monique L. Den Boer^{1,4}

¹Department of Pediatric Oncology, Erasmus University Medical Center-Sophia Children's Hospital, Rotterdam, The Netherlands; ²Department of Human Genetics, Radboud University Medical Centre and Nijmegen Centre for Molecular Life Sciences, Nijmegen, The Netherlands; ³Research Institute for Oncology, Radboud University Medical Centre, Nijmegen, The Netherlands; ⁴Dutch Childhood Oncology Group, The Hague, The Netherlands; ⁵Leukemia Research Cytogenetics Group, Newcastle University, Newcastle upon Tyne, United Kingdom; ⁶Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN; ⁷Department of Immunology, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁸Laboratory of Pediatric Oncology, Department of Pediatrics, Radboud University Medical Centre and Nijmegen Centre for Molecular Life Sciences, Nijmegen, The Netherlands; ⁹Cooperative Acute Lymphoblastic Leukemia Study Group, Research Centre and Clinic of Pediatric Oncology, University Medical Center Eppendorf, Hamburg, Germany; and ¹⁰Department of Biostatistics, Erasmus University Medical Center, Rotterdam, The Netherlands

Key Points

- *BCR-ABL1*-like signature and *IKZF1* deletions are clinically important to identify high-risk acute lymphoblastic patients.

Most relapses in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) are not predicted using current prognostic features. Here, we determined the co-occurrence and independent prognostic relevance of 3 recently identified prognostic features: *BCR-ABL1*-like gene signature, deletions in *IKZF1*, and high *CRLF2* messenger RNA expression (*CRLF2*-high). These features were determined in 4 trials representing 1128 children with ALL: DCOG ALL-8, ALL9, ALL10, and Cooperative ALL (COALL)-97/03. *BCR-ABL1*-like, *IKZF1*-deleted, and *CRLF2*-high cases constitute 33.7% of *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL cases, of which *BCR-ABL1*-like and *IKZF1* deletion (co)occurred most frequently. Higher cumulative incidence of relapse was found for *BCR-ABL1*-like and *IKZF1*-deleted, but not *CRLF2*-high, cases relative to remaining BCP-ALL cases, reflecting the observations in each of the cohorts analyzed separately. No relapses occurred among cases with *CRLF2*-high as single feature, whereas 62.9% of all relapses in *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL occurred in cases with *BCR-ABL1*-like signature and/or *IKZF1* deletion. Both the *BCR-ABL1*-like signature and *IKZF1* deletions were prognostic features independent of conventional prognostic markers in a multivariate model, and both remained prognostic among cases with intermediate minimal residual disease. The *BCR-ABL1*-like signature and an *IKZF1* deletion, but not *CRLF2*-high, are prognostic factors and are clinically of importance to identify high-risk patients who require more intensive and/or alternative therapies. (*Blood*. 2013;122(15):2622-2629)

Introduction

The 5-year event-free survival (EFS) rate of childhood acute lymphoblastic leukemia (ALL) currently exceeds 80%.¹ This is attributed to risk-adjusted treatment, which implements risk factors such as an early treatment response and cytogenetic abnormalities in leukemic cells, as well as the implementation of rationally designed phases in the treatment backbone of ALL including remission induction, central nervous system prophylaxis, and intensification and maintenance phases of treatment. More than half of the children in whom contemporary therapies failed were initially classified as non-high risk,² highlighting the need for improved prognostic markers.

The IKAROS transcription factor, encoded by *IKZF1*, was recently associated with an unfavorable prognosis in childhood

B-cell precursor (BCP) ALL.³⁻⁶ A monoallelic (often partial) deletion in *IKZF1* results in a loss of its tumor suppressor function.^{7,8} *EBF1*, *MSH2*, and *MCL1* were demonstrated as target genes for IKAROS.⁹ These genes contribute to the development of B cells (*EBF1*), DNA repair (*MSH2*), and survival/antiapoptosis (*MCL1*). Deletions in *IKZF1* aborted the activation of these genes, which may facilitate B-cell leukemogenesis.⁹ Ten percent to 15% of BCP-ALL cases have an *IKZF1* deletion, which therefore represents the most frequently observed genetic marker for an unfavorable outcome identified in children.³⁻⁶

The *BCR-ABL1*-like gene expression signature is a second recently identified unfavorable prognostic marker in childhood BCP-ALL.

Submitted October 15, 2012; accepted June 10, 2013. Prepublished online as *Blood* First Edition paper, August 23, 2013; DOI 10.1182/blood-2012-10-462358.

A.v.d.V., E.W., R.P.K., and M.L.D.B. contributed equally to this study.

The data reported in this article have been deposited in the Gene Expression Omnibus database (accession number GSE13351).

The online version of this article contains a data supplement.

There is an Inside *Blood* commentary on this article in this issue.

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BCR-ABL1-like ALL was identified based on a gene expression signature of its leukemic cells, which is similar to that of *BCR-ABL1*-positive ALL, although these leukemic cells do not harbor the *BCR-ABL1* translocation.^{5,10-12} Approximately 15% of BCP-ALL cases have *BCR-ABL1-like* ALL, which is associated with a 5-year EFS of <60%, similar to that observed in *BCR-ABL1*-positive BCP-ALL.^{5,10-12} More than 80% of *BCR-ABL1-like* ALL cases have abnormalities in genes involved in B-cell development, amongst others. *IKZF1* deletions in ~40%.^{5,10-12}

A third recently identified adverse marker is linked to increased expression of cytokine receptor-like factor 2 (*CRLF2*).¹³⁻¹⁶ *CRLF2* and interleukin-7 receptor α (*IL7RA*) form the thymic stromal-derived lymphopoietin receptor, which promotes B-cell progenitor proliferation by activating the Janus kinase (Jak) 2–signal transducer and activator of transcription 5 pathway.¹⁶ The high expression of *CRLF2* in BCP-ALL arises either via a cryptic deletion, which juxtaposes *CRLF2* to the *P2RY8* promoter (*P2RY8-CRLF2*), or from the positioning of *CRLF2* under control of the immunoglobulin heavy chain locus (*IGH@-CRLF2*) enhancer element.^{16,17} In addition, *CRLF2* activity can be increased by gain-of-function mutations either in *CRLF2* itself¹⁸ or in its partner gene, *IL7RA*.¹⁹ High *CRLF2* messenger RNA (mRNA) expression (*CRLF2*-high) occurs in 5% to 14% of childhood BCP-ALL cases, and its incidence is remarkably high in Down syndrome ALL (50% to 55%).^{14,17,20}

The presence of the aforementioned features was determined in several studies that used different inclusion and outcome criteria and a variety of techniques. Therefore, the relationship between these features, as well as their prognostic independence within a given patient population, remains unclear. Here, we studied these 3 molecular features in 1128 cases from 4 independent cohorts of children with newly diagnosed ALL. This study shows that both the *BCR-ABL1-like* signature and *IKZF1* deletions are strong and independent prognostic features, whereas *CRLF2*-high is not uniformly predictive for an unfavorable outcome.

Methods

Detailed methods are described in the supplemental Appendix (see the *Blood* Web site).

Patients and leukemic cell samples

This study comprised 1128 children with newly diagnosed ALL in 3 consecutive Dutch Childhood Oncology Group trials (DCOG ALL-8, ALL-9, and ALL-10)²¹ and 2 German Cooperative ALL trials (COALL 06-97 and 07-03)²² that were combined for analysis and are referred to hereafter as COALL-97/03. Patients were stratified into low, intermediate, and high risk according to stratification markers in the treatment protocol (DCOG ALL-8,²¹ ALL-9,²¹ ALL10 [Supplemental Table 1], COALL 06-97,²² and 07-03²²). In accordance with the Declaration of Helsinki, written informed consent was obtained from parents or guardians, and institutional review boards approved the use of excess diagnostic material for research purposes. Minimal residual disease (MRD) of DCOG ALL-10 patients was diagnosed by Sanquin (Amsterdam) and Erasmus University Medical Center (MC) Rotterdam.²³ Cases were screened for hyperdiploidy (>50 chromosomes or DNA index ≥ 1.16), *ETV6-RUNX1* and *BCR-ABL1* fusion products, and rearrangements of *TCF3* and *MLL* by routine diagnostic procedures; complementary data were generated by the Erasmus MC laboratory. Cases negative for these cytogenetic markers were assigned to the B-other group (supplemental Figure 1).

BCR-ABL1-like signature

New *BCR-ABL1-like* cases were identified using gene expression profiling of leukemic cells with Affymetrix U133 plus 2.0 microarrays. The same set

of 110 gene probes and clustering procedure were used to identify novel *BCR-ABL1-like* cases in a newly arrayed cohort of 572 BCP-ALL cases using the previous study cohort of 107 DCOG cases as a reference (supplemental Figure 2; supplemental Table 2; Gene Expression Omnibus accession number GSE13351).¹⁰ Newly arrayed cases that hierarchically clustered together with previously identified *BCR-ABL1-like* and *BCR-ABL1*-positive cases were identified as new *BCR-ABL1-like* cases if proved negative for the *BCR-ABL1* translocation (supplemental Figure 2; supplemental Data).¹⁰

IKZF1 deletions and mutations

IKZF1 deletions identified using the SALSA P335 ALL-*IKZF1* Multiplex Ligation-dependent Probe Amplification (MLPA) assay (MRC-Holland) were confirmed using the P202 MLPA assay, a previously described in-house *IKZF1* MLPA probe set,⁴ or comparative genomic hybridization analysis (SurePrint G3 180K array; Agilent).¹⁰ The presence of inactivating mutations in *IKZF1* exons 4, 5, 6, and 8 was measured using Sanger sequencing in *BCR-ABL1-like* cases.^{4,5}

Abnormalities in *CRLF2* by genetic lesions and gene expression

P2RY8-CRLF2 and *IGH@-CRLF2* (detected by interphase fluorescence in situ hybridization)¹⁶ are linked to high *CRLF2* mRNA expression levels as determined by Affymetrix gene expression microarrays (supplemental Figure 3).²⁴ Those cases with a signal intensity of the Affymetrix probe set 208303_s_at above the 90th percentile of the total BCP-ALL group were classified as *CRLF2*-high.

Statistics

Categorical and continuous variables were analyzed using nonparametric statistical tests. The cumulative incidence of relapse (CIR) with death as a competing event was calculated as described by Fine and Gray,²⁵ considering relapse and nonresponse to induction chemotherapy as events. EFS rates were determined using Cox's univariate and multivariate proportional hazard analyses with relapse, nonresponse, and death by leukemia considered as events. Differences with a *P* value < .05 were considered significant.

Results

A total of 1128 newly diagnosed children with BCP-ALL (*n* = 971) and T-lineage ALL (T-ALL; *n* = 157) were analyzed for the *BCR-ABL1-like* signature, *IKZF1* deletions, and/or high *CRLF2* expression.

The DCOG ALL-10 study subset was highly representative of the entire cohort of eligible cases, whereas the other 3 subsets had higher white blood cell (WBC) counts than the cases in each of the trial cohorts (supplemental Table 3). To prevent bias from known high-risk factors in the outcome analyses, CIR and Cox's EFS analyses were analyzed in BCP-ALL patients after excluding *BCR-ABL1*-positive and *MLL*-rearranged cases (supplemental Figure 1).

Frequency and cumulative relapse incidences of BCP-ALL with *BCR-ABL1-like* expression signature

Ninety-four (16%) new *BCR-ABL1-like* cases were identified among the total of 572 BCP-ALL with gene expression data (Table 1; supplemental Figure 2). *BCR-ABL1-like* cases were identified in 52% (30/58) of DCOG ALL-10 B-other cases (ie, negative for hyperdiploidy, *ETV6-RUNX1* fusion, *TCF3* rearrangement, *MLL* rearrangement, or *BCR-ABL1* translocation). Similar frequencies were observed in the other cohorts (50% ALL-8 [16/32], 64% ALL-9 [25/39], and 49% COALL-97/03 [23/47]). None of the T-ALL patients were found to carry the *BCR-ABL1-like* signature. *BCR-ABL1-like* cases were more often assigned to intermediate- and

Table 1. Frequency of new prognostic features in subtypes of pediatric ALL

Subgroup	<i>BCR-ABL1-like</i>		Subtype	<i>IKZF1-deleted</i>		<i>CRLF2-high</i>	
	n	%		n	%	n	%
Hyperdiploid	0/130	0		32/208	15	25/130	19
<i>ETV6-RUNX1</i> positive	0/162	0		7/225	3	0/162	0
<i>TCF3</i> rearranged	0/19	0		1/19	5	0/19	0
<i>MLL</i> rearranged	0/12	0		2/27	7	1/12	8
B-other*	94/176	53	<i>BCR-ABL1-like</i> †	37/92	40	15/94	16
			Remaining B-other	16/80	20	10/82	12
<i>BCR-ABL1</i> positive	0/24	0		16/23	70	0/24	0
Unknown BCP-ALL‡	0/49	0		43/233	18	5/49	10
Total BCP-ALL	94/572	16		154/907	17	56/572	10
T-ALL	0/80	0		5/157	3	1/80	1

*B-other cases are negative for hyperdiploidy, *ETV6-RUNX1*, *TCF3* rearrangement, *MLL* rearrangement, and *BCR-ABL1* translocation.

†*BCR-ABL1-like* cases are negatively tested for *BCR-ABL1* translocation; *BCR-ABL1*-positive cases are allocated to the *BCR-ABL1*-positive group.

‡Unknown BCP-ALL cases are negative for *BCR-ABL1* translocation, *MLL* rearrangement, and *BCR-ABL1-like* signature, but other cytogenetic lesions were not determined.

high-risk treatment than other BCP-ALL cases as a consequence of higher WBC count or age at diagnosis (supplemental Tables 6 and 7).

The 94 *BCR-ABL1-like* ALL had a 5-year CIR of 32% (95% confidence interval [CI] of 27% to 37%) compared with 11% (95% CI of 9% to 12%) for the 442 *BCR-ABL1*-negative/*MLL* wild-type BCP-ALL cases ($P < .001$; Figure 1A; supplemental Table 8). Similar significant differences were measured within each of the 4 study cohorts (supplemental Table 9; supplemental Figure 5). *BCR-ABL1-like* patients who were treated in the standard-risk ($P = .05$) and medium-risk ($P = .06$) arms of the most recent DCOG ALL-10 study exhibited a trend toward an unfavorable outcome (supplemental Figure 9).

Frequency and cumulative relapse incidences of *IKZF1*-deleted BCP-ALL

IKZF1 deletions were identified in 16% (136/857) of *BCR-ABL1*-negative and *MLL* wild-type BCP-ALL cases (Table 1). Whole-gene deletions encompassing *IKZF1* exons 1 to 8 accounted for 40.4% of these deleted cases. In the remaining cases, partial deletions of exons 4 to 7 (26.5% of cases), exons 2 to 7 (9.6%), exons 4 to 8 (7.3%), exons 2 to 3 (8.1%), exons 2 to 8 (4.4%), and other exons (3.7%; supplemental Figure 4) were detected. Whole-gene or partial deletions in the protein-coding exons 2 to 8 were considered to compose 1 biological group, as reported previously.⁴⁻⁶

The highest incidence of *IKZF1* deletions occurred in *BCR-ABL1*-positive (70%; 16/23) and *BCR-ABL1-like* (40%; 37/92) cases (Table 1). *IKZF1* deletions were also identified in other BCP-ALL

types, including hyperdiploid (15% of cases), *TCF3*-rearranged (5%), *ETV6-RUNX1*-positive (3%), *MLL*-rearranged (7%), and remaining B-other ALL cases (20%). Three percent of T-ALL cases (5/157) contained an *IKZF1* deletion (Table 1).

The 136 *IKZF1*-deleted BCP-ALL cases had a 5-year CIR of 34% (95% CI of 30% to 38%) compared with 13% (95% CI of 12% to 15%) in the 721 nondeleted *BCR-ABL1*-negative/*MLL* wild-type cases ($P < .001$; Figure 1B; supplemental Table 8). Each treatment protocol exhibited similar prognostic differences, except for DCOG ALL-8–treated cases (supplemental Table 9; supplemental Figure 6). A deletion in *IKZF1* was primarily predictive of poor outcome in patients treated in the medium-risk arm ($P = .008$) of the DCOG ALL-10 protocol (supplemental Figure 9). The most discriminating prognostic values for *IKZF1* deletions were observed in hyperdiploid ($P = .04$) and non-*BCR-ABL1-like* B-other ($P = .02$) cases, whereas no prognostic difference was observed for *IKZF1* deletions in the *BCR-ABL1-like* and *ETV6-RUNX1*-positive genetic subtypes in the DCOG ALL-10 study (Figure 2). Inactivating mutations in exons 4 to 6 and 8 were not identified in *BCR-ABL1-like*, *IKZF1* wild-type cases.

Frequency and cumulative relapse incidences of BCP-ALL with high *CRLF2* mRNA expression levels

High *CRLF2* mRNA expression, irrespective of *CRLF2*-rearrangement status, was found in hyperdiploid (19% of cases), *BCR-ABL1-like* (16%), *MLL*-rearranged (8%), and B-other (12%) cases; whereas no *CRLF2*-high cases were observed in *ETV6-RUNX1*-positive,

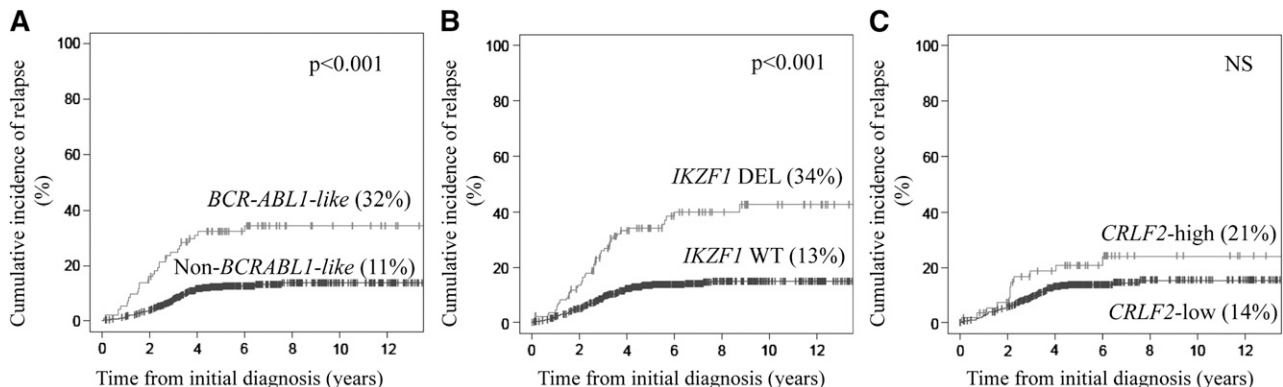


Figure 1. CIR for the *BCR-ABL1-like* expression signature, *IKZF1*-deleted, and high *CRLF2* mRNA expression in newly diagnosed children with *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL. CIR with death as a competing event was calculated using the method of Fine and Gray²⁵ in a pooled analysis of all 4 study cohorts and plotted against the time from initial diagnosis. For each feature, CIR at the 5-year follow-up is given in parentheses. (A) CIR of 94 *BCR-ABL1-like* and 442 non-*BCR-ABL1-like* BCP-ALL cases. (B) CIR of 136 *IKZF1*-deleted (DEL) and 721 *IKZF1* wild-type (WT) cases. (C) Comparison between 55 cases with high *CRLF2* expression and 481 cases with low *CRLF2* expression.

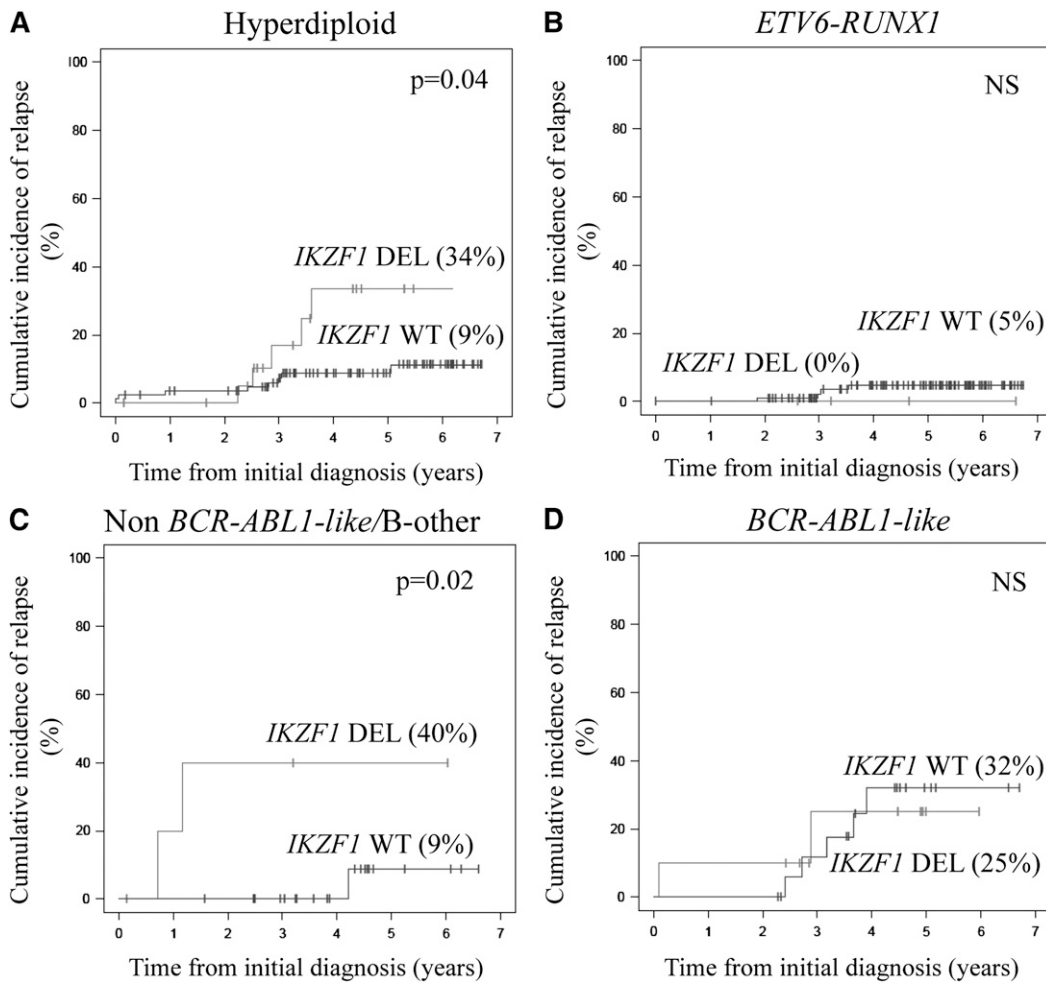


Figure 2. CIR for *IKZF1* status among subtypes of ALL. CIR with death as a competing event was calculated for the DCOG ALL-10 study cases with (A) high hyperdiploid ALL (>50 chromosomes) including 21 *IKZF1*-deleted and 87 wild-type cases; (B) *ETV6-RUNX1*-positive ALL including 4 *IKZF1*-deleted and 100 wild-type cases; (C) non-*BCR-ABL1*-like B-other ALL including 5 *IKZF1*-deleted and 22 wild-type cases; and (D) *BCR-ABL1*-like ALL including 10 *IKZF1*-deleted and 19 wild-type cases. For each feature, the CIR at 5 years of follow-up is indicated in parentheses.

BCR-ABL1-positive, or *TCF3*-rearranged ALL (Table 1). High *CRLF2* mRNA expression was observed in 1% (1/80) of T-ALL cases.

No statistically significant difference in CIR was found for *CRLF2* mRNA expression status using the 90th percentile cutoff value in a pooled analysis of all patients or in the individual treatment cohorts, except for COALL-97/03 ($P = .007$; Figure 1C; supplemental Figure 7; supplemental Tables 8 and 9). The CIR analyses did not reveal a significant difference if the 92.5 and the 95th percentiles were used as cutoff values for high *CRLF2* mRNA expression, nor did the CIR differ between cases with and without genetic *CRLF2* aberration (*IGH@-CRLF2* and *P2RY8-CRLF2*; supplemental Figure 8). In addition, *CRLF2*-high (90th percentile cutoff) had no prognostic value within the subset of *BCR-ABL1*-like cases (supplemental Figure 12).

Univariate and multivariate EFS analyses of BCP-ALL with *BCR-ABL1*-like signature, *IKZF1* deletion, and/or *CRLF2*-high expression

The results of the CIR analyses of *BCR-ABL1*-like, *IKZF1*-deleted, and *CRLF2*-high cases among the cohort of 507 *BCR-ABL1*-negative/*MLL* wild-type BCP-ALL patients for which all 3 features were determined (supplemental Table 4; supplemental Figure 10) were similar to those obtained by separate analyses of larger sample

sets of *BCR-ABL1*-like signature (n = 536), *IKZF1* status (n = 857), and *CRLF2* expression (n = 536; Figure 1). The 3 molecular features together composed 33.7% of BCP-ALL cases (Figure 3A), of which the *BCR-ABL1*-like signature and *IKZF1* deletions were the most prominent both as single lesions and in combination (Figure 3B; supplemental Table 5A). No relapses were predicted by the 14.6% of cases with high *CRLF2* expression alone. In contrast, 62.9% (44/70) of all relapses in BCP-ALL (negative for *BCR-ABL1* and *MLL* wild type) occurred in cases with the *BCR-ABL1*-like signature and/or an *IKZF1* deletion. Of these 44 relapsed cases, 30 had a *BCR-ABL1*-like signature and 25 an *IKZF1* deletion, and thus 11 cases had both a *BCR-ABL1*-like signature and an *IKZF1* deletion (Figure 3C; supplemental Table 5B).

Univariate analysis of EFS of the 507 patients stratified according to treatment revealed that the *BCR-ABL1*-like signature (hazard ratio [HR] 3.5, 95% CI 2.2-5.7, $P < .001$), *IKZF1* deletions (HR 2.8, 95% CI 1.7-4.6, $P < .001$) and WBC count of ≥ 50 cells per nL (HR 2.3, 95% CI 1.4-3.8, $P = .001$) were associated with an unfavorable prognosis; whereas high *CRLF2* expression, age, and gender were not (supplemental Table 10A). Multivariate analysis stratified according to treatment protocol suggested that the *BCR-ABL1*-like signature, a high WBC count, and male gender had independent prognostic value (HR 3.1, 95% CI 1.8-5.2, $P < .001$; HR 1.8, 95% CI 1.1-3.1, $P = .02$; HR 1.8, 95% CI 1.1-3.0, $P = .02$, respectively).

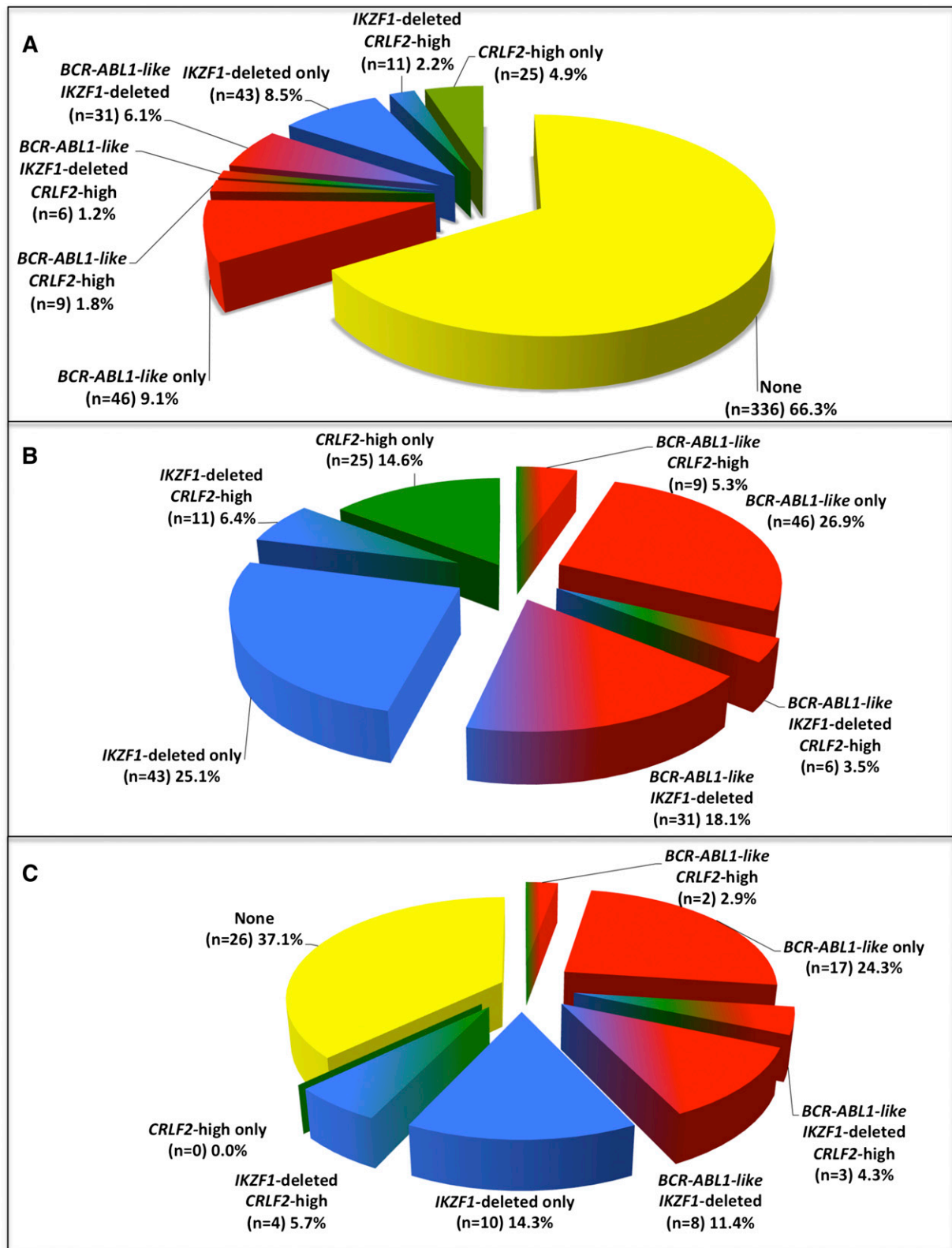
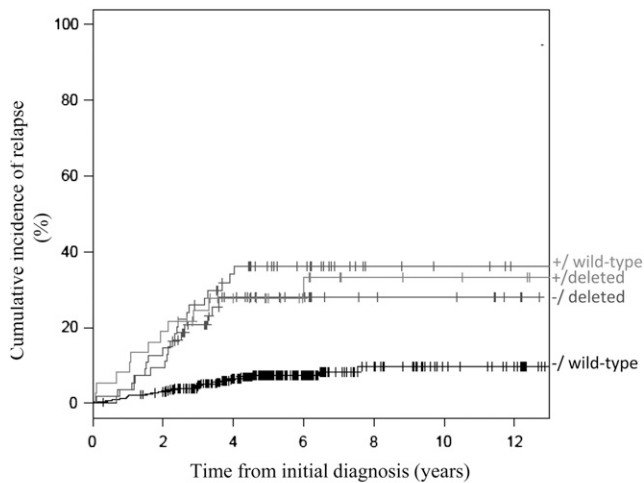


Figure 3. Frequency of *BCR-ABL1-like*, *IKZF1-deleted*, and *CRLF2-high* mRNA expression features among children with *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL. (A) Distribution of cases with *BCR-ABL1-like*, *IKZF1-deleted*, and/or *CRLF2-high* mRNA expression in 507 children with *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL. See also supplemental Table 5A. (B) Pie chart depicting the distribution of patients carrying 1 or more of the aforementioned features. Collectively, these 3 features constitute 33.7% of *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL cases as shown in panel A. See also supplemental Table 5A. (C) Pie chart depicting the percentages of relapsed patients carrying 1, 2, or 3 features. In total, 62.9% of all relapsed cases were associated with *BCR-ABL1-like* and/or *IKZF1-deleted* features. See also supplemental Table 5B.



<i>BCR-ABL1-like</i>	<i>IKZF1</i>	n	5-year CIR	<i>p</i> -value
+	wild-type	55	36%	<0.001
+	deleted	37	27%	<0.001
-	deleted	54	28%	<0.001
-	wild-type	361	7%	-

Figure 4. Interaction between *BCR-ABL1-like* and *IKZF1* status for predicting relapse. CIR with death as a competing event for cases with or without a deletion in *IKZF1* among *BCR-ABL1-like* and non-*BCR-ABL1-like* ALL cases. The box below the graphs indicates the 5-year CIR, and *P* values compared with the reference group of non-*BCR-ABL1-like* and *IKZF1* wild-type cases (black line). *BCR-ABL1*-positive and *MLL*-rearranged cases were excluded from this analysis.

In a similar model applied to *IKZF1* status, deletions in *IKZF1* (HR 2.5, 95% CI 1.5-4.2, *P* = .001), high WBC count (HR 2.0, 95% CI 1.4-3.4, *P* = .007), and male gender (HR 1.7, 95% CI 1.1-2.9, *P* = .03) were significant. High *CRLF2* expression did not have prognostic value in this multivariate approach (supplemental Table 10B).

Interestingly, among the *BCR-ABL1-like* cases, CIR was not affected by the *IKZF1* deletion status. Conversely, CIR among the *IKZF1*-deleted cases was not affected by the *BCR-ABL1-like* signature (Figure 4). A multivariate interaction model including both *BCR-ABL1-like* signature and *IKZF1* status showed that the HRs for *BCR-ABL1-like* ALL alone (HR 5.3, 95% CI 2.9-9.7), *IKZF1*-deleted cases alone (HR 4.4, 95% CI 2.3-8.4), or combined *BCR-ABL1-like* signature and *IKZF1*-deleted cases (HR 3.7, 95% CI 2.0-8.4) were similarly adverse for each of these categories compared with cases without the *BCR-ABL1-like* signature and nondeleted *IKZF1* (*P* < .001, treatment-stratified analysis; supplemental Table 10C).

Relationship between MRD status and *BCR-ABL1-like* ALL, *IKZF1*-deleted cases, and *CRLF2*-high cases in DCOG ALL-10 study

MRD levels were prospectively measured for eligible DCOG ALL-10 cases and used to stratify patient risk (see supplemental Appendix). All 3 MRD categories contained *BCR-ABL1-like*, *IKZF1*-deleted, and/or *CRLF2*-high cases, but the distribution was skewed toward the MRD-intermediate and MRD-high categories for both *BCR-ABL1-like* (*P* = .001) and *IKZF1*-deleted (*P* = .03) cases relative to control BCP-ALL cases (supplemental Table 6). More precisely, 17.9% (5/28), 6.7% (2/30), and 5.6% (1/18) of the *BCR-ABL1-like*, *IKZF1*-deleted, and *CRLF2*-high BCP-ALL cases, respectively, were assigned to the MRD-high category (supplemental

Table 11), collectively, representing 5 distinct BCP-ALL cases. Remarkably, *BCR-ABL1-like* was the common denominator of these 5 MRD-high cases, although this finding is preliminary (supplemental Table 11). Both the *BCR-ABL1-like* signature (HR 3.7, 95% CI 1.2-11.5, *P* = .026) and *IKZF1*-deleted cases (HR 2.7, 95% CI 1.0-7.0, *P* = .043), but not *CRLF2*-high cases, remained predictive of a poor clinical outcome among the MRD-intermediate BCP-ALL cases (supplemental Table 12).

Discussion

Recently, new molecular features were discovered that can predict an unfavorable outcome in childhood BCP-ALL and include a *BCR-ABL1-like* gene expression signature,^{10,12} a (partial) deletion in *IKZF1*,³⁻⁶ and high expression of *CRLF2*.¹³⁻¹⁶ These factors are predictive among cases that do not exhibit known poor-prognostic features and therefore have potential clinical value. However, to date, these leukemia cell characteristics have been assessed separately in discrete patient cohorts with different inclusion criteria, limiting an analysis of their independent prognostic relevance. Moreover, variable technical approaches and cutoff values for positivity have hampered comparisons between published reports.

This is the first study to analyze the prognostic value and interaction between these 3 recently identified molecular features using standardized conditions and uniform inclusion criteria for newly diagnosed childhood ALL patients enrolled in 4 different treatment protocols. We show that 16%, 17%, and 10% of BCP-ALL cases have the *BCR-ABL1-like* signature, *IKZF1* deletions, and high *CRLF2* mRNA expression, respectively. These frequencies are consistent with previous observations in single-marker studies in BCP-ALL.^{4-6,10,12,15}

A relatively high frequency of *IKZF1* deletions was found in *BCR-ABL1-like* cases identified in the present study (40%) and those described by the St. Jude Children’s Research Hospital and Children’s Oncology Group (80% to 90%), although the technique for the identification of *BCR-ABL1-like* cases was different.^{11,12} The St. Jude Children’s Research Hospital and Children’s Oncology Group studies identified the *BCR-ABL1-like* (or Ph-like) cases by using gene expression profiling and gene set enrichment analysis,⁵ a recognition of outliers by sampling ends clustering approach (so-called R8 cluster),^{11,12} or a prediction analysis for microarrays classifier model.^{11,26} These approaches used a dynamic, nonfixed classifier, which differed per study. In the present study, we used a fixed model using our previously identified 110 gene probes and the same identical hierarchical clustering method as in our discovery study.¹⁰ A second difference is that the St. Jude Children’s Research Hospital and Children’s Oncology Group studies analyzed patients enrolled in high-risk protocols (eg, P9906 and AALL0232), whereas in the present study the *BCR-ABL1-like* cases were identified in both standard-, medium-, and high-risk arms. In the present more population-based study, the *BCR-ABL1-like* signature, *IKZF1* deletions, and *CRLF2*-high cases together constitute 33.7% of *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL cases, of which concomitant *BCR-ABL1-like* signature and *IKZF1* deletion was the most frequent (Figure 3).

The *BCR-ABL1-like* and *IKZF1*-deleted features were predictive of an unfavorable prognosis in most studied treatment protocols, including the MRD-based DCOG ALL-10 study. This indicates that *BCR-ABL1-like* and *IKZF1*-deleted cases are not yet recognized by other means and that no sufficient risk-adapted treatment is provided in contemporary regimens. Correspondingly, multivariate analysis revealed that the adverse prognoses of *BCR-ABL1-like* signature and

IKZF1-deleted cases are independent of the conventional risk factors and remained predictive for an unfavorable outcome in the group of patients with intermediate MRD levels. This highlights the value of both features as strong independent risk factors. Importantly, both *BCR-ABL1-like* and *IKZF1*-deleted cases were identified across all stratification arms and were either exclusively (*BCR-ABL1-like*) or primarily (*IKZF1* deletion) present in the B-other category, for which new prognostic markers are urgently needed (Table 1).

The biology underlying *BCR-ABL1-like* ALL is largely unknown, but there is a relatively high frequency of deletions in B-cell development genes (eg, *IKZF1*, *EBF1*, *PAX5*, and *VPREB1*) and in cell cycle genes (*CDKN2A/2B*), whereas mutations and translocations are mainly observed in genes associated with cytokine signaling (eg, *CRLF2*, *IL7R*, and *EPOR*) and kinase pathways (eg, *ABL1* and *JAK2*).^{5,10-12,26,27} The genomic deletions are most often recurrent (eg, *IKZF1* deletion), whereas the mutations and translocations most often affect single genes and different fusion partners. Exceptions are recurrent lesions found for *CRLF2* and *JAK*-family genes and to a lesser extent for the *EBF1-PDGFRB* translocation in an independent validation cohort.^{11,27} In the present cohort of BCP-ALL cases, we infrequently found mutations and translocations affecting *JAK2* (<2%). These lesions were mainly found in non-*BCR-ABL1-like* cases with high *CRLF2* expression and represent only a minority of our *CRLF2*-high cases (J. Marchante, M.L.D.B., unpublished observation). This low frequency of *JAK2* abnormalities, therefore, cannot explain the poor prognosis ascribed to the *BCR-ABL1-like* signature or an *IKZF1* deletion in the present study. The *ABL1*-translocated cells were shown to be sensitive to the *ABL1* kinase domain inhibitors imatinib and dasatinib, whereas *JAK2*-affected leukemic cells were sensitive to the *JAK*-inhibitor ruxolitinib.^{11,28} Although no common genomic denominator has yet been identified for *BCR-ABL1-like* ALL, screening of patients for the presence of *ABL1* and *JAK*-family lesions may identify *BCR-ABL1-like* patients who may benefit from implementing targeted tyrosine kinase inhibitors in their treatment.

IKZF1 deletions were observed in every ALL subtype, including at low frequency in T-ALL, and primarily showed unfavorable prognostic value in hyperdiploid and non-*BCR-ABL1-like* B-other cases (Figure 2; supplemental Figure 11). Together, this indicates that a broad range of ALL types are affected by aberrant IKAROS function. *IKZF1* deletions contribute to uncontrolled proliferation in *BCR-ABL1*-positive ALL,^{7,8} most likely by interfering with *c-Myb* and *Bmi1*-regulated transcription of B-cell differentiation genes.^{29,30} Interestingly, *IKZF1* deletions had no additive adverse effect on the prognosis of *BCR-ABL1-like* cases (Figure 4). Moreover, the unfavorable prognosis of *BCR-ABL1-like* cases without *IKZF1* deletion could not be explained by the presence of inactivating mutations in *IKZF1*. The role of aberrant IKAROS in *BCR-ABL1-like* and other ALL subtypes is yet unclear, and the current findings underscore the importance of elucidating these mechanisms. One intriguing observation is that expression of the immunoglobulin joining chain (*IGJ*) gene is deregulated in both *IKZF1*-deleted and *BCR-ABL1-like* ALL.^{10,12} The immunoglobulin joining chain is involved in immunoglobulin maturation,³¹⁻³³ whereas IKAROS controls the processing of immature B-cell receptors into immunoglobulins.^{34,35} Whether *IKZF1* deletions functionally affect this maturation process clearly warrants further investigation.

In this study, high *CRLF2* mRNA expression was the weakest prognostic feature, being predictive of an unfavorable outcome in COALL-97/03 but not in the 3 DCOG-trials. The group with high *CRLF2* mRNA expression included all cases with genetic *CRLF2* aberrations (supplemental Figure 3), which is in correspondence with previous observations.¹⁵ We cannot rule out that high *CRLF2*

expression will show some prognostic relevance when larger sample sizes are used. We therefore do not want to state that deregulated *CRLF2* completely lacks prognostic relevance in pediatric BCP-ALL, but we want to emphasize that the discriminative prognostic value of high *CRLF2* mRNA expression is lower than that of the *BCR-ABL1-like* signature and *IKZF1* deletion. The prognostic and clinical value of *CRLF2* expression levels in childhood BCP-ALL is controversial and appears to be dependent on the treatment protocol: predictive for adverse outcome in US and German (eg, COALL97/03 in the present study),^{15,24,36,37} but not Dutch (DCOG ALL-8, ALL-9, and ALL-10) and British treatment protocols.^{14,37} Other possible explanations of this discrepancy include differences in methodology (eg, mRNA expression vs genomic rearrangement) and inclusion criteria (eg, inclusion of cases with Down syndrome or cases of Hispanic/Latino ethnicity)^{17,24} and the possibility that the treatment and/or stratification criteria could mitigate any adverse effects of high *CRLF2* expression. Moreover, we observed that *CRLF2*-high cases that lacked *IKZF1* deletions and/or the *BCR-ABL1-like* signature did not relapse, suggesting that the prognostic value attributed to this factor may arise from the co-occurrence of *IKZF1* deletions and/or the *BCR-ABL1-like* signature.¹³⁻¹⁶

We conclude that the *BCR-ABL1-like* signature and *IKZF1* deletions in BCP-ALL cells are strong independent prognostic features that can identify patients at high risk for treatment failure, even among cases with intermediate MRD levels. In contrast, high *CRLF2* mRNA expression lacks such discriminative prognostic power in our studies. These findings highlight the important clinical value of these 2 molecular features for identifying patients at high risk for treatment failure who might otherwise remain unrecognized. The implementation of *BCR-ABL1-like* signature as new risk factor in DCOG trials is being considered, and to this aim, a feasibility study for gene expression profiling in clinical practice has been initiated. Because the *IKZF1* deletion can be detected using DNA, which is less vulnerable to degradation than mRNA, this genomic marker can be more easily implemented in clinical practice. In the recently initiated DCOG ALL-11 protocol, it was therefore decided to first implement the *IKZF1* status as new risk factor. In the DCOG ALL-11 trial, medium-risk patients with a deletion in *IKZF1* will receive an additional year of maintenance therapy.

Acknowledgments

This work was supported by the Dutch Cancer Society (KWF; grants Erasmus MC Rotterdam 2007-3718 [M.L.D.B., R.P.] and KUN 2009-4298 [R.P.K., P.M.H.]); the Sophia Foundation for Scientific Research (SSWO; grant 2010-138) (A.v.d.V., M.L.D.B.); the Pediatric Oncology Foundation Rotterdam (M.L.D.B., R.P.); the National Institutes of Health National Cancer Institute (research grant R37 CA36401) (W.E.E.); St. Jude Children's Research Hospital, Memphis, TN (W.E.E.); and the Kay Kendal Leukaemia Fund, United Kingdom (L.J.R., C.J.H.).

These funding sources had no role in the collection, analysis, or interpretation of the results, or in writing the manuscript and the decision to submit this manuscript.

Authorship

Contribution: A.v.d.V., R.P., R.P.K., and M.L.D.B. designed the study; A.v.d.V., M.E.W., W.E.E., and M.L.D.B. performed the gene expression studies to identify *BCR-ABL1-like* and

CRLF2-high expressing patients; A.v.d.V., E.W., M.E.W., S.V.V.R., F.V.L., R.P.K., and M.L.D.B. performed the MLPA analyses, genomic polymerase chain reaction, and array-comparative genomic hybridization to identify and validate deletions in *IKZF1*; M.E.W., L.J.R., and C.J.H. performed *CRLF2* fluorescence in situ hybridization analyses; P.M.H., H.A.D.G.-K., G.E., M.A.H., and E.S. collected and provided patient characteristics and clinical responses; V.H.J.v.d.V. was responsible for the MRD analyses;

A.v.d.V., L.M.K., H.A.D.G.-K., and D.R. contributed to the statistical analyses of the data; and all authors read, revised, and approved the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Monique L. Den Boer, Erasmus MC-Sophia Children's Hospital, Doctor Molewaterplein 60, 3015GJ Rotterdam, The Netherlands; e-mail: m.l.denboer@erasmusmc.nl.

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