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IKZF1plus Defines a New Minimal Residual Disease–Dependent Very-Poor Prognostic Profile in Pediatric B-Cell Precursor Acute Lymphoblastic Leukemia

Martin Stanulla, Elif Dagdan, Marketa Zaliova, Anja Möricke ...+34 more authors

Institutions: Hannover Medical School

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IKZF1 Defines a New Minimal Residual Disease-Dependent Very-Poor Prognostic Profile in Pediatric B-Cell Precursor Acute Lymphoblastic Leukemia

Stanulla, Martin ; Dagdan, Elif ; Zaliova, Marketa ; et al ; Bourquin, Jean-Pierre ; Bornhauser, Beat

Abstract: Purpose Somatic deletions that affect the lymphoid transcription factor-coding gene IKZF1 have previously been reported as independently associated with a poor prognosis in pediatric B-cell precursor (BCP) acute lymphoblastic leukemia (ALL). We have now refined the prognostic strength of IKZF1 deletions by analyzing the effect of co-occurring deletions. Patients and Methods The analysis involved 991 patients with BCP ALL treated in the Associazione Italiana Ematologia ed Oncologia Pediatrica-Berlin-Frankfurt-Muenster (AIEOP-BFM) ALL 2000 trial with complete information for copy number alterations of IKZF1, PAX5, ETV6, RB1, BTG1, EBF1, CDKN2A, CDKN2B, Xp22.33/Yp11.31 (PAR1 region; CRLF2, CSF2RA, and IL3RA), and ERG; replication of findings involved 417 patients from the same trial. Results IKZF1 deletions that co-occurred with deletions in CDKN2A, CDKN2B, PAX5, or PAR1 in the absence of ERG deletion conferred the worst outcome and, consequently, were grouped as IKZF1. The IKZF1 group comprised 6% of patients with BCP ALL, with a 5-year event-free survival of 53 ± 6% compared with 79 ± 5% in patients with IKZF1 deletion who did not fulfill the IKZF1 definition and 87 ± 1% in patients who lacked an IKZF1 deletion (P .001). Respective 5-year cumulative relapse incidence rates were 44 ± 6%, 11 ± 4%, and 10 ± 1% (P .001). Results were confirmed in the replication cohort, and multivariable analyses demonstrated independence of IKZF1. The IKZF1 prognostic effect differed dramatically in analyses stratified by minimal residual disease (MRD) levels after induction treatment: 5-year event-free survival for MRD standard-risk IKZF1 patients was 94 ± 5% versus 40 ± 10% in MRD intermediate- and 30 ± 14% in high-risk IKZF1 patients (P .001). Corresponding 5-year cumulative incidence of relapse rates were 6 ± 6%, 60 ± 10%, and 60 ± 17% (P .001). Conclusion IKZF1 describes a new MRD-dependent very-poor prognostic profile in BCP ALL. Because current AIEOP-BFM treatment is largely ineffective for MRD-positive IKZF1 patients, new experimental treatment approaches will be evaluated in our upcoming trial AIEOP-BFM ALL 2017.

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ORIGINAL REPORT

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Martin Stanulla, Elif Dagdan, Marketa Zaliova, Anja Möricke, Chiara Palmi, Giovanni Cazzaniga, Cornelia Eckert, Geertruy te Kronnie, Jean-Pierre Bourquin, Beat Bornhauser, Rolf Koehler, Claus R. Bartram, Wolf-Dieter Ludwig, Kirsten Bleckmann, Stefanie Groeneveld-Krentz, Denis Schewe, Stefanie V. Junk, Laura Hinze, Norman Klein, Christian P. Kratz, Andrea Biondi, Arndt Borkhardt, Andreas Kulozik, Martina U. Muckenthaler, Giuseppe Basso, Maria Grazia Valsecchi, Shai Izraeli, Britt-Sabina Petersen, Andre Franke, Petra Dörge, Doris Steinemann, Oskar A. Haas, Renate Panzer-Grümayer, Hélène Cavé, Richard S. Houlston, Gunnar Cario, Martin Schrappe, and Martin Zimmermann, for the TRANSCALL Consortium and the International BFM Study Group

Author affiliations and support information (if applicable) appear at the end of this article.

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M. Stanulla, E.D., G.C., M. Schrappe, and M. Zimmermann contributed equally to this work.

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Corresponding author: Martin Stanulla, MD, Department of Pediatric Hematology and Oncology, Hannover Medical School, Carl-Neuberg-Str 1, D-30625 Hannover, Germany; e-mail: stanulla.martin@mh-hannover.de.

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ABSTRACT

Purpose
Somatic deletions that affect the lymphoid transcription factor–coding gene *IKZF1* have previously been reported as independently associated with a poor prognosis in pediatric B-cell precursor (BCP) acute lymphoblastic leukemia (ALL). We have now refined the prognostic strength of *IKZF1* deletions by analyzing the effect of co-occurring deletions.

Patients and Methods
The analysis involved 991 patients with BCP ALL treated in the Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster (AIEOP-BFM) ALL 2000 trial with complete information for copy number alterations of *IKZF1*, *PAX5*, *ETV6*, *RB1*, *BTG1*, *EBF1*, *CDKN2A*, *CDKN2B*, Xp22.33/Yp11.31 (PAR1 region; *CRLF2*, *CSF2RA*, and *IL3RA*), and *ERG*; replication of findings involved 417 patients from the same trial.

Results
IKZF1 deletions that co-occurred with deletions in *CDKN2A*, *CDKN2B*, *PAX5*, or PAR1 in the absence of *ERG* deletion conferred the worst outcome and, consequently, were grouped as *IKZF1*^{plus}. The *IKZF1*^{plus} group comprised 6% of patients with BCP ALL, with a 5-year event-free survival of 53 ± 6% compared with 79 ± 5% in patients with *IKZF1* deletion who did not fulfill the *IKZF1*^{plus} definition and 87 ± 1% in patients who lacked an *IKZF1* deletion ($P \leq .001$). Respective 5-year cumulative relapse incidence rates were 44 ± 6%, 11 ± 4%, and 10 ± 1% ($P \leq .001$). Results were confirmed in the replication cohort, and multivariable analyses demonstrated independence of *IKZF1*^{plus}. The *IKZF1*^{plus} prognostic effect differed dramatically in analyses stratified by minimal residual disease (MRD) levels after induction treatment: 5-year event-free survival for MRD standard-risk *IKZF1*^{plus} patients was 94 ± 5% versus 40 ± 10% in MRD intermediate- and 30 ± 14% in high-risk *IKZF1*^{plus} patients ($P \leq .001$). Corresponding 5-year cumulative incidence of relapse rates were 6 ± 6%, 60 ± 10%, and 60 ± 17% ($P \leq .001$).

Conclusion
IKZF1^{plus} describes a new MRD-dependent very-poor prognostic profile in BCP ALL. Because current AIEOP-BFM treatment is largely ineffective for MRD-positive *IKZF1*^{plus} patients, new experimental treatment approaches will be evaluated in our upcoming trial AIEOP-BFM ALL 2017.

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ASSOCIATED CONTENT

Appendix
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Data Supplement
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INTRODUCTION

More than 80% of pediatric patients with B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) treated with modern protocols can achieve a long-term cure, but a significant proportion of these patients still experience relapse and therapy-related toxicities.[1,2] Thus, the focus of therapy improvement for children with ALL—and any childhood cancer—is not only cure but also a minimization of both short- and long-term therapy-associated toxicities.[3,4] These goals can be achieved with better personalized adjustment

of therapy to the perceived risk of relapse.[5,6] At least 50% of relapses occur among patients classified as having standard or intermediate risk.[7,8] Although relapse-prone patients within these low-risk groups require more-effective therapy, a large number of the remaining children are most likely overtreated with toxic chemotherapy. Therefore, a pressing medical need exists for new prognostic markers to improve risk assessment and tailor treatment in this group of children with ALL.

Triggered by these clinical needs, technical advances in the field of genomic analyses have stimulated a large number of studies aimed at finding new biomarkers relevant for risk stratification.[9-22] A number of candidate markers have been identified and suggested as potentially useful for risk stratification in childhood ALL. To date, however, most of these genetic or genomic markers are not used regularly to support decision making on current clinical protocols for childhood ALL. General reasons for this lack of translation include the following: Initial studies have been conducted on small study populations only; study populations have been selected according to the availability of leukemic specimens for analysis; studies have been in patient populations that received heterogeneous and partly outdated treatments; conflicting results have been observed in various studies; and methodological differences in marker assessment and uncertainties about assay procedures exist. Furthermore, when assessed on up-to-date Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster (AIEOP-BFM) ALL protocols, the prognostic strength of many new high-risk markers is limited, which makes exposure to more-intensive and -toxic treatments difficult to justify for the potential benefit of a minority of patients identified by the marker.

The *IKZF1* gene encodes Ikaros, a zinc-finger transcription factor required for the development of all lymphoid lineages.[23] Somatic deletions of *IKZF1* have been described as a new high-risk marker in BCP ALL.[24-31] Recently, we and others demonstrated that activation of JAK-STAT signaling may enhance, whereas deletions of *ERG* can attenuate, the negative prognostic effect conferred by *IKZF1* deletions in BCP ALL.[28,32-36] The current study refines the prognostic strength of *IKZF1* deletions and characterizes a new very-poor prognostic subgroup termed *IKZF1*^{plus}. *IKZF1*^{plus} is a combination of previously described gene deletions and provides additional proof for the importance of moving from prognostic factors to prognostic profiles to sharpen the predictive strength of newly described genetic markers for integration into risk-adapted innovative treatment strategies for pediatric ALL.

PATIENTS AND METHODS

Sample Selection

The study included 991 patients with BCP ALL diagnosed between August 1999 and May 2009 who were enrolled into the international multicenter trial AIEOP-BFM ALL 2000 for the treatment of pediatric ALL in Germany and had complete information available for copy number alterations of *IKZF1*, *PAX5*, *ETV6*, *RB1*, *BTG1*, *EBF1*, *CDKN2A*, *CDKN2B*, Xp22.33/Yp11.31 (PAR1 region; *CRLF2*, *CSF2RA*, and *IL3RA* genes), and *ERG*.[8,27,37] For independent replication, 417 patients with BCP ALL treated in Italy in the same trial and with comparable genetic data available were analyzed (Fig 1).

In AIEOP-BFM ALL 2000, patients were stratified into three risk groups (standard, intermediate, and high). Risk group stratification included minimal residual disease (MRD) analysis and required two MRD targets with sensitivities of $\leq 1 \times 10^{-4}$. MRD standard-risk (MRD-SR) patients were MRD negative on treatment days 33 and 78, and MRD high-risk (MRD-HR) patients had residual disease of $\geq 5 \times 10^{-4}$ on treatment day 78. All remaining results were considered MRD intermediate risk (MRD-IR).[37] Although MRD analysis was the main stratification criterion in AIEOP-BFM ALL 2000, established high-risk parameters also were retained as follows: Patients with prednisone poor response (Table 1), $\geq 5\%$ leukemic blasts in the bone marrow on day 33, or positivity for t(4;11) or its molecular equivalent (*MLL-AF4* gene fusion) were stratified into the high-risk group independent of their MRD results. Treatment included the use of standard drugs (eg, prednisone, vincristine, daunorubicin, L-asparaginase, cyclophosphamide, ifosfamide, cytarabine, 6-mercaptopurine, 6-thioguanine, and methotrexate) and in some of the patients, cranial irradiation and/or hematopoietic stem-cell transplantation (high-risk patients only). For analysis stratified according to National Cancer Institute (NCI) criteria, the definition of standard risk was an age at diagnosis of 1 year to younger than 10 years and an initial WBC count of $< 50{,}000/\mu L$; NCI high-risk patients were 10 years and older and/or had an initial WBC of $\geq 50{,}000/\mu L$. The institutional review boards of Hannover Medical School and all participating centers approved the study. Informed consent was obtained in accordance with the Declaration of Helsinki.

Molecular Genetic Analyses

Copy number alterations of *IKZF1*, *PAX5*, *ETV6*, *RB1*, *BTG1*, *EBF1*, *CDKN2A*, *CDKN2B*, and the Xp22.33/Yp11.31 region (PAR1 region; *CRLF2*, *CSF2RA*, and *IL3RA* genes; only deletion of *CSF2RA* and *IL3RA* and retention of the *CRLF2* probe associated with *P2RY8-CRLF2* fusion that leads to overexpression of *CRLF2* was regarded as PAR1 deletion in this study) were assessed by multiplex ligation-dependent probe amplification (MLPA) analyses (SALSA MLPA P335 ALL and P202-A1 IKZF1 kits; MRC-Holland, Amsterdam, the Netherlands). *ERG* deletion detection was performed by a custom-made multiplex polymerase chain reaction assay as described previously.[27,36]

Statistical Analysis

Differences in the distribution of individual parameters among patient subsets were analyzed using the χ^2 or Fisher's exact test for categorical variables and the Mann-Whitney *U* test for continuous variables. Event-free survival (EFS) was defined as the time from diagnosis to the date of last follow-up in complete remission (censored time) or first event. Events were resistance to therapy (nonresponse), relapse, secondary neoplasm, or death as a result of any cause. Failure to achieve remission as a result of early death or nonresponse was considered as event at time zero. The Kaplan-Meier method was used to estimate survival rates; differences were compared using the log-rank test (two-sided).[38,39] Cumulative incidence functions for competing events were estimated according to Kalbfleisch and Prentice[40] and were compared with Gray's test.[41] The Cox proportional hazards regression model was used to estimate hazard ratios and their 95% CIs for prognostic factors.[42] Furthermore, the classification and regression tree (CART) method was used to divide successively the *IKZF1*-deleted cohort into prognostic subgroups, which split a node into two subgroups using the covariate that best discriminated the cumulative incidence of relapse (CIR) on the basis of the lower limit of a one-sided 5% CI for the risk ratio.[43,44] The variable with the highest lower limit was used for the split. The process stopped when no covariate could split subgroups farther. Statistical analyses were conducted with SAS-PC 9.1 software (SAS Institute, Cary, NC). $P \leq .05$ was considered statistically significant.

Development of Prognostic IKZF1 Deletion Profile

The prognostic effects of *IKZF1* deletion in combination with deletions in either *PAX5*, *ETV6*, *RB1*, *BTG1*, *EBF1*, *CDKN2A*, *CDKN2B*, or PAR1 were analyzed by separate assessments of CIRs for patients with

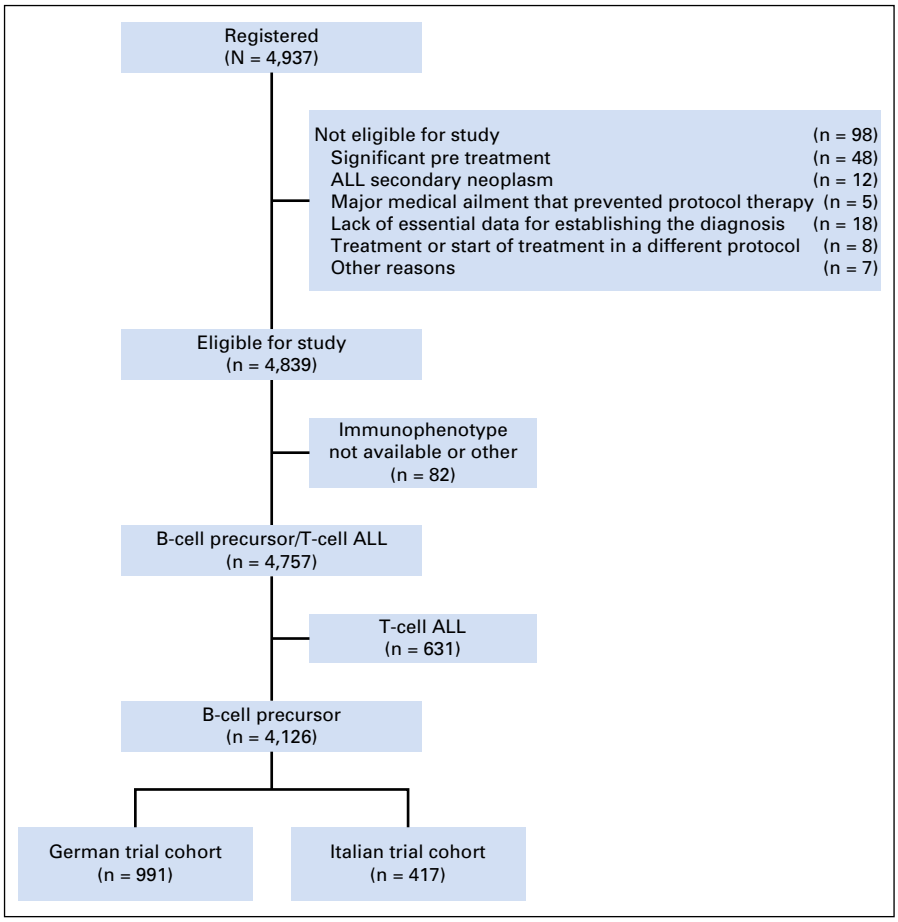

Fig 1. Flow diagram of patients in trial AIEOP-BFM (Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) ALL 2000. ALL, acute lymphoblastic leukemia.

IKZF1 deletion compared with those with *IKZF1* deletion co-occurring with one of the deletions mentioned. When numbers within a deletion subgroup exceeded 10 patients each, heterozygous and homozygous co-occurrence of a respective deletion with *IKZF1* deletion were addressed separately as well. When numbers were too low or the incidence of relapse was similar in patients with heterozygous and homozygous deletions, these groups were combined. Combinations that demonstrated a statistically significant higher CIR than *IKZF1* deletion alone in a single analysis qualified for integration into the prognostic deletion profile. Deletion selection for profile integration was further supported using CART analysis to identify deletions that best separate patients with *IKZF1* deletions into risk groups with adverse prognosis.[43,44]

RESULTS

Initially, we analyzed the association between *IKZF1* deletion and other recurrent deletions observed in ALL by using MLPA data from 146 of the 991 patients whose ALL cells carried *IKZF1* deletions. Combined effects on relapse incidence were evaluated for deletions of *IKZF1* with either *PAX5*, *ETV6*, *RB1*, *BTG1*, *EBF1*, *CDKN2A*, *CDKN2B*, or those that affected the PAR1 region and led to *CRLF2* overexpression (for definition of deletions that affect the PAR1 region, see Patients and Methods, under Molecular Genetic Analyses). *IKZF1* deletions that co-occurred with deletions in *CDKN2A*, *CDKN2B*, *PAX5*, or the PAR1 region conferred lower EFS and higher CIRs than their respective negative counterparts (Appendix Fig A1, online only). For *CDKN2A*, a negative effect was detectable for heterozygous as well as homozygous deletions, whereas for *CDKN2B*, only homozygous deletions were of additive value (Appendix Fig A1). Consequently, all categories of *CDKN2A*, *CDKN2B*, *PAX5*, or the PAR1 region associated with a dismal prognosis in the context of *IKZF1* deletion were grouped. Deletion selection for grouping was further supported by CART analysis (Appendix Fig A2, online only). The additional markers assessed by MLPA (*ETV6*, *RB1*, *BTG1*, and *EBF1*) and evaluated for combination effects were not included for refining *IKZF1* status because no significant influence on outcome was detectable (Appendix Fig A3, online only). Deletions of *ERG* attenuate the negative prognostic effect conferred by *IKZF1* deletions.[35,36] Within the group of BCP ALL that carry an *IKZF1* deletion and at least one additional unfavorable deletion in *CDKN2A*, *CDKN2B*, *PAX5*, or the PAR1 region, five patients demonstrated an *ERG* deletion as well. Thus, only minimal overlap was observed between the described aberration profile and deletion of *ERG*. Of note, none of these five patients experienced a relapse. The frequency and distribution of *IKZF1* and co-occurring deletions with prognostic effect are listed in Appendix Table A1 (online only). Consequently, the final definition of the new prognostic profile termed *IKZF1*^{plus} was as follows: deletion of *IKZF1* that co-occurred with at least one additional deletion in *CDKN2A*, *CDKN2B* (homozygous

Table 1. Clinical Characteristics According to *IKZF1* Status in 991 Patients With B-Cell Precursor ALL Treated in Germany in Trial AIEOP-BFM ALL 2000

Characteristic	*IKZF1* Status, No. (%)			*P**
	No *IKZF1* Deletion	*IKZF1* Deletion	*IKZF1*^{plus}	
No. of patients	845	83	63	
Sex				
Male	447 (52.9)	44 (53.0)	30 (47.6)	
Female	398 (47.1)	39 (47.0)	32 (52.4)	.718
Age at diagnosis, years				
< 10	665 (78.7)	44 (53.0)	39 (61.9)	
≥ 10	180 (21.3)	39 (47.0)	24 (38.1)	< .001
Presenting WBC, count/μL				
< 10,000	383 (45.3)	29 (34.9)	10 (15.9)	
10,000 to < 50,000	308 (36.4)	38 (45.8)	23 (36.5)	
50,000 to < 100,000	93 (11.0)	9 (10.8)	17 (27.0)	
≥ 100,000	61 (7.2)	7 (8.4)	13 (20.6)	< .001
CNS positivity†				
No	788 (93.3)	77 (92.8)	55 (87.3)	
Yes	21 (2.5)	3 (3.6)	7 (11.1)	< .001
Unknown	36 (4.3)	3 (3.6)	1 (1.5)	
Hyperdiploidy‡				
No	457 (54.8)	53 (63.9)	48 (76.2)	
Yes	114 (13.5)	14 (16.9)	4 (6.4)	.091
Unknown	274 (32.4)	16 (19.3)	11 (17.5)	
ETV6-RUNX1				
Negative	587 (69.5)	77 (92.8)	61 (96.8)	
Positive	218 (25.8)	3 (3.6)	1 (1.6)	< .001
Unknown	40 (4.7)	3 (3.6)	1 (1.6)	
MLL-AF4				
Negative	840 (99.4)	83 (100)	63 (100)	
Positive	5 (0.6)	0 (0.0)	0 (0.0)	.649
Prednisone response§				
Good	790 (93.5)	68 (81.9)	56 (88.9)	
Poor	52 (6.2)	13 (15.7)	7 (11.1)	.001
Unknown	3 (0.4)	2 (2.4)	0 (0.0)	
MRD risk group‖				
Standard	338 (40.0)	15 (18.1)	19 (30.2)	
Intermediate	343 (40.6)	37 (44.6)	27 (42.9)	
High	28 (3.3)	20 (24.1)	10 (15.9)	< .001
Unknown	136 (16.1)	11 (13.3)	7 (11.1)	
Final risk group¶				
Standard	329 (38.9)	13 (15.7)	19 (30.2)	
Intermediate	438 (51.8)	45 (54.2)	33 (52.4)	
High	78 (9.2)	25 (30.1)	11 (17.5)	< .001
GATA3 rs3824662 genotype				
GG	206 (24.4)	23 (27.7)	13 (20.6)	
TG	136 (16.1)	17 (20.5)	20 (31.7)	
TT	20 (2.4)	4 (4.8)	6 (9.5)	.029
Unknown	483 (57.2)	39 (47.0)	24 (38.1)	

NOTE. *IKZF1*^{plus} definition: presence of *IKZF1* deletion and at least an additional deletion in *PAX5*, *CDKN2A*, *CDKN2B*, or PAR1 in the absence of *ERG* deletion. *IKZF1* deletion definition: *IKZF1* deletion present but does not fulfill the *IKZF1*^{plus} definition.

Abbreviations: AIEOP-BFM, Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; MRD, minimal residual disease.

*χ^2 or Fisher's exact test that compares the groups with various *IKZF1* statuses.

†Puncture nontraumatic, WBC count > 5/μL CSF with identifiable blasts.

‡Defined by cytogenetics (> 50 chromosomes) or by flow cytometric analyses of the ratio of DNA content of leukemic G0/G1 cells to normal diploid lymphocytes (≥ 1.16).

§Good, < 1,000 leukemic blood blasts/μL on treatment day 8; poor, ≥ 1,000/μL.

‖Standard risk, negative on treatment days 33 and 78; high risk, leukemic cell load ≥ 5×10^{-4} on treatment day 78; all other results intermediate risk.

¶Treatment group (for definition see Patients and Methods, under Sample Selection).

deletion only), *PAX5*, or PAR1 in the absence of *ERG* deletion. Characteristics of *IKZF1*^{plus} compared with *IKZF1* deletions that did not fulfill the *IKZF1*^{plus} definition (hereafter called *IKZF1* deletion) as well as no *IKZF1* deletion are listed in Table 1. Altogether, *IKZF1*^{plus} comprised 6% of patients with BCP ALL. Specifically, patients with *IKZF1*^{plus} positivity had higher WBC counts at diagnosis, were more often positive for CNS disease, and were rarely *ETV6-RUNX1* positive. Their prednisone response and MRD kinetics were worse than for those who lacked any *IKZF1* deletion (Table 1). In an analysis that incorporated 498 patients with available information, the germline *GATA3* single-nucleotide variant rs3824662, which formerly was related to the risk of developing B-other or Philadelphia-like pediatric ALL, was associated with the *IKZF1*^{plus} group (Table 1).[45,46]

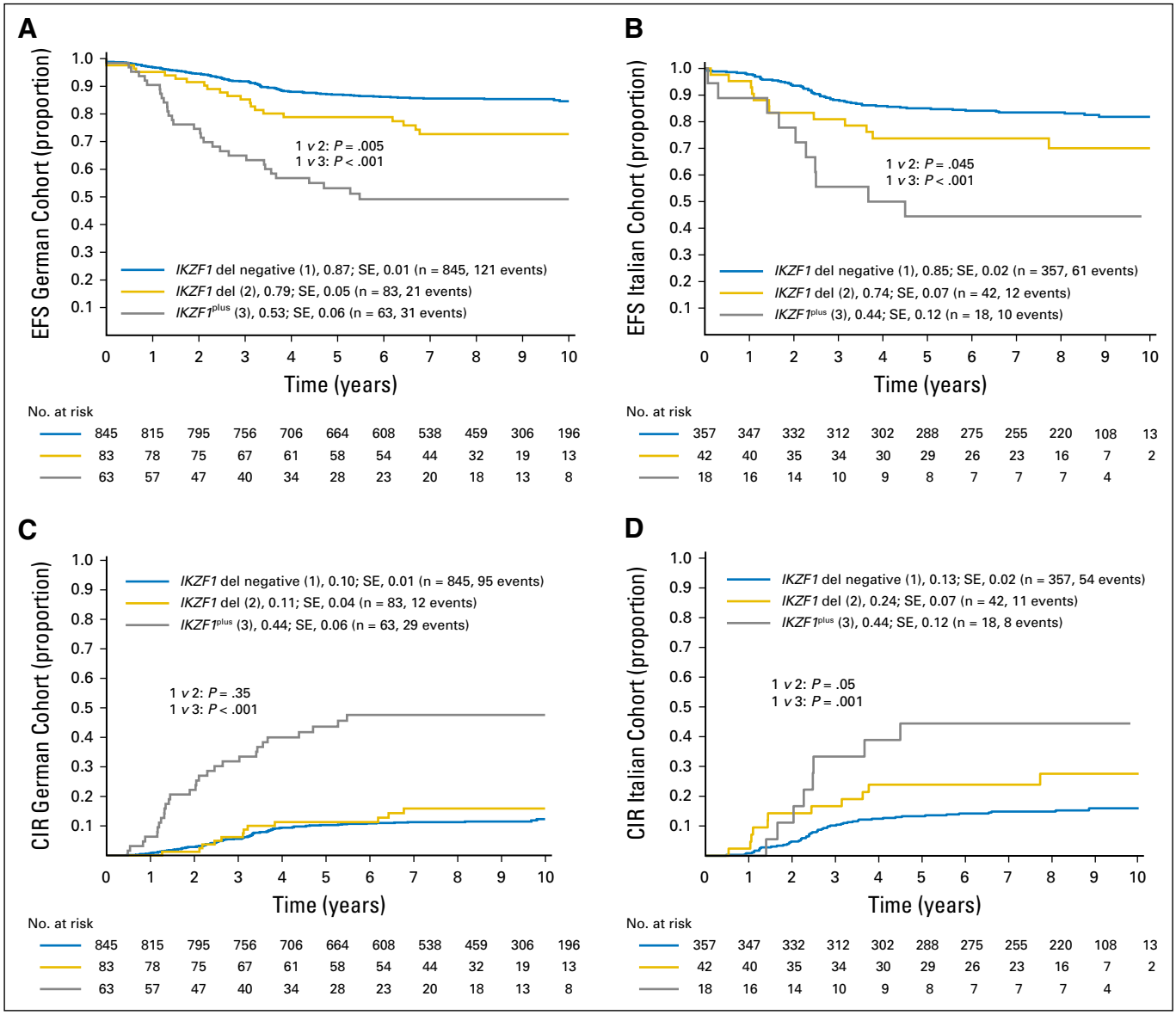

Fig 2. Event-free survival (EFS) and cumulative incidence of relapse (CIR) at 5 years according to *IKZF1* status (no *IKZF1* deletion [*IKZF1* del], *IKZF1* del, *IKZF1*^{plus}) in patients with B-cell precursor acute lymphoblastic leukemia (ALL) treated in trial AIEOP-BFM (Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) ALL 2000. (A) EFS in 991 patients from the observational cohort recruited in Germany. (B) EFS in 417 patients from the replication cohort recruited in Italy. (C) CIR in the observational cohort. (D) CIR in the replication cohort. del, deletion.

In outcome analysis, *IKZF1*^{plus} was associated with a 5-year EFS of 53 ± 6% compared with 79 ± 5% in patients with *IKZF1* deletion or 87 ± 1% in patients who lacked any *IKZF1* deletion (*IKZF1*^{plus} *v* no *IKZF1* deletion, $P \leq .001$; Fig 2A). The respective 5-year CIRs were 44 ± 6%, 11 ± 4%, and 10 ± 1% (*IKZF1*^{plus} *v* no *IKZF1* deletion, $P \leq .001$; Fig 2C). Appendix Fig A4 (online only) shows the poor prognostic effect of *IKZF1*^{plus} in analyses stratified by NCI risk group and patients with high initial WBC counts of ≥ 50,000/μL and ≥ 100,000/μL. Multivariable analyses that included variables of clinical and prognostic relevance previously described in AIEOP-BFM ALL 2000[37] demonstrated independence of *IKZF1*^{plus}—with *IKZF1*^{plus} conferring the largest hazard ratio for relapse—and underscored its strong prognostic effect (Table 2).

We next used an independent cohort of 417 patients treated in AIEOP-BFM ALL 2000 in Italy who had the necessary genetic information available for confirmation of the negative prognostic effect of *IKZF1*^{plus} (Appendix Table A2, online only). The 5-year EFS observed for *IKZF1*^{plus} was 44 ± 12% compared with 74 ± 7% for *IKZF1* deletions or 85 ± 2% for no *IKZF1* deletions (*IKZF1*^{plus} *v* no *IKZF1* deletion, $P \leq .001$; Fig 2B). The respective 5-year CIRs were 44 ± 12%, 24 ± 7%, and 13 ± 2% (*IKZF1*^{plus} *v* no *IKZF1* deletion, $P = .001$; Fig 2D). Similar to the observation cohort, multivariable analyses in the validation cohort demonstrated independence of *IKZF1*^{plus} (Appendix Table A3, online only).

We have previously demonstrated that MRD risk group (for definition, see Patients and Methods, under Sample Selection) is

Table 2. Estimated HRs From the Multivariable Cox Proportional Hazards Model on Event-Free Survival and Hazard of Relapse in Patients With B-Cell Precursor ALL From Trial AIEOP-BFM ALL 2000

Variable	Event			Relapse		
	HR	95% CI	*P*	HR	95% CI	*P*
IKZF1^{plus}*	2.62	1.41 to 4.86	.002	4.00	1.91 to 8.37	< .001
IKZF1 deletion†	1.03	0.60 to 1.76	.928	0.85	0.44 to 1.67	.639
ETV6-RUNX1 positivity‡	0.97	0.60 to 1.56	.903	1.03	0.62 to 1.70	.908
MRD standard risk§	0.49	0.31 to 0.77	.002	0.42	0.26 to 0.68	< .001
MRD high risk§	3.42	2.07 to 5.65	< .001	2.04	1.10 to 3.81	.024
Slow early MRD response‖	2.70	1.64 to 4.44	< .001	2.69	1.60 to 4.53	< .001
Poor prednisone response¶	1.13	0.68 to 1.88	.633	0.94	0.51 to 1.72	.840
Presenting WBC# count ≥ 100,000/μL	2.36	1.49 to 3.74	< .001	2.59	1.56 to 4.28	< .001

Abbreviations: AIEOP-BFM, Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; HR, hazard ratio; MRD, minimal residual disease.

*Presence of *IKZF1* deletion and at least an additional deletion in *PAX5*, *CDKN2A*, *CDKN2B*, or PAR1 in the absence of *ERG* deletion. HR compared with patients who lacked an *IKZF1* deletion.

†*IKZF1* deletion present but does not fulfill the *IKZF1*^{plus} definition. HR compared with patients who lacked an *IKZF1* deletion.

‡Compared with *ETV6-RUNX1* negativity.

§MRD standard risk, negative on treatment days 33 and 78; MRD high risk, leukemic cell load ≥ 5×10^{-4} on treatment day 78; all other results MRD intermediate risk. HR compared with other MRD groups.

‖MRD ≥ 5×10^{-4} on treatment day 33 and positivity of < 5×10^{-4} on treatment day 78. HR compared with MRD intermediate-risk patients with no slow early response.

¶Leukemic blasts ≥ 1,000/μL in the peripheral blood on treatment day 8. HR compared with patients with < 1,000/μL leukemic blasts.

#HR compared with patients with presenting WBC counts < 100,000/μL.

the strongest prognostic factor in our protocol.[37] Therefore, we assessed the prognostic effect of *IKZF1*^{plus} in the context of treatment response and treatment intensity. Unexpected dramatic differences among MRD risk groups were detected in these analyses: The 5-year EFS for MRD-SR *IKZF1*^{plus} patients was 94 ± 5% compared with 40 ± 10% in MRD-IR and 30 ± 14% in MRD-HR *IKZF1*^{plus} patients; the corresponding 5-year CIRs were 6 ± 6%, 60 ± 10%, and 60 ± 17%. Figure 3 demonstrates these results for *IKZF1*^{plus} compared with *IKZF1* deletions and no *IKZF1* deletions. When the type and number of deletions in *IKZF1*^{plus} were assessed by MRD risk group to explain the observed differential prognostic effect (Appendix Tables A4 and A5, online only), neither obvious differences in type nor significant differences in number of deletions (P = .750) were detected among the MRD risk groups. Finally, we analyzed whether we could detect changes in aberration profiles of *IKZF1*^{plus} samples from initial diagnosis to relapse. For this purpose, we compared 14 samples with MLPA analyses from both time points and did not identify recurrent profile changes (Appendix Table A6, online only). Taken together, *IKZF1*^{plus} conferred an extremely high risk of relapse, specifically to MRD-IR and MRD-HR patients.

DISCUSSION

We define a new and powerful prognostic profile, *IKZF1*^{plus}, for pediatric ALL treated with BFM therapy-based clinical protocols. The use of *IKFZ1* deletion in combination with specific additional single genetic deletions provides an independent strong molecular stratification marker in addition to MRD measurements. The extremely poor outcome of patients treated by either intermediate- or high-intensity chemotherapy suggests that MRD-IR and MRD-HR *IKZF1*^{plus} patients should be enrolled in clinical trials that incorporate and evaluate experimental therapies.

Research on the clinical and biologic aspects of ALL has identified numerous prognostic factors used in modern clinical protocols to stratify patients according to their probability of treatment failure into risk groups of various treatment intensities (eg, standard/low, intermediate, high).[1,2] During the past two decades, molecular monitoring of MRD in AIEOP-BFM studies has become the most important prognostic factor.[8,37] However, although a poor early response to therapy as characterized by our MRD-HR definition predicts treatment failure and defines a true high-risk patient population, the majority of recurrences are still observed in the large group of MRD-IR patients. In AIEOP-BFM ALL 2000, 69% of BCP ALL relapses occurred in these patients, which exemplifies that for a majority of patients with disease recurrence, the precedent treatment stratification strategy does not adequately reflect their actual risk of relapse.[8,37] Consequently, current treatment stratification still needs improvement to lead to a more-precise early characterization of the patient at true risk of relapse. Upfront identification of these patients is essential in future clinical trials for optimal therapy early on and for high-risk patients, the timely introduction of innovative treatment options, such as immunotherapy.[47] *IKZF1*^{plus} represents a simple pragmatic approach to addressing this dilemma: The group comprised approximately 6% of patients with BCP ALL, which accounts for approximately 25% of relapses; the majority of the relapses occurred in MRD-IR patients. Therefore, the use of this new biomarker is directly applicable to clinical practice to improve risk stratification of patients treated in AIEOP-BFM ALL trials.

Several studies have linked *IKZF1* deletions to an unfavorable clinical outcome of BCP ALL in various treatment protocols, including those that used risk stratification guided by MRD monitoring.[24-36,48-51] As a consequence, international study groups have included *IKZF1* deletion status in their high-risk treatment stratification strategies for patients with BCP ALL.[48,49] However, other study groups, including AIEOP-BFM, currently do not

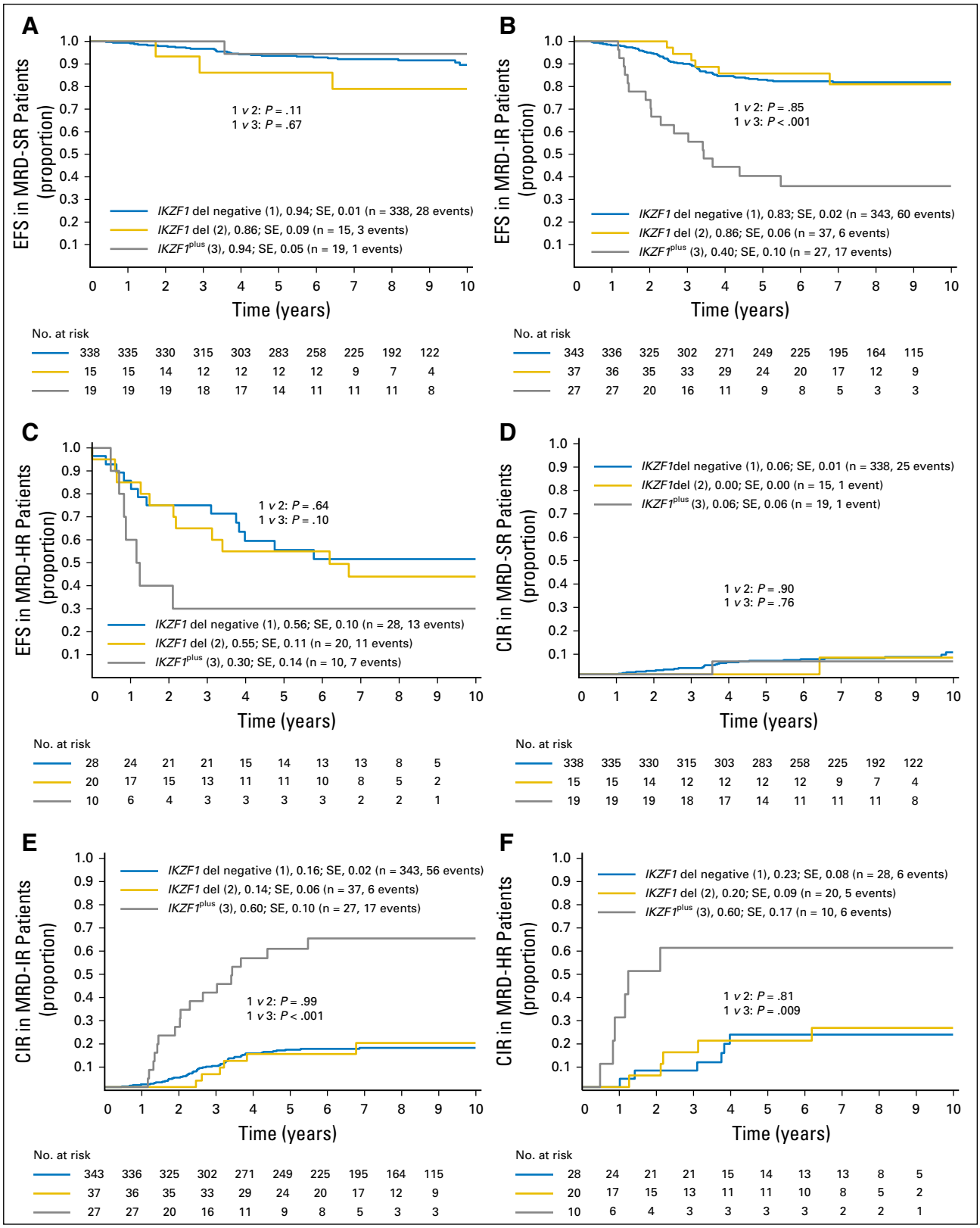

Fig 3. Event-free survival (EFS) and cumulative incidence of relapse (CIR) at 5 years according to *IKZF1* status (no *IKZF1* deletion [*IKZF1* del], *IKZF1* del, *IKZF1*^{plus}) in 837 patients with B-cell precursor acute lymphoblastic leukemia (ALL) with available minimal residual disease (MRD) data treated in trial AIEOP-BFM (Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) ALL 2000. MRD standard-risk (MRD-SR) patients were MRD negative on treatment days 33 and 78; MRD high-risk (MRD-HR) patients had leukemic cell loads of $\geq 5 \times 10^{-4}$ on treatment day 78; and all other results were classified as MRD intermediate risk (MRD-IR). For exact definitions, see Patients and Methods, under Sample Selection. (A) EFS in MRD-SR patients. (B) EFS in MRD-IR patients. (C) EFS in MRD-HR patients. (D) CIR in MRD-SR patients. (E) CIR in MRD-IR patients. (F) CIR in MRD-HR patients. del, deletion.

follow this strategy because the prognostic effect of *IKZF1* deletions is judged as not profound enough to justify exposure to the toxic adverse effects of high-risk treatment of a majority of actual intermediate-risk patients not in need of treatment intensification.[27,33,52] By the definition of *IKZF1*^{plus}, we refined the prognostic strength of *IKZF1* deletions by describing a very-poor prognostic *IKZF1* deletion–associated genetic aberration profile that now, in combination with MRD analyses, justifies clinical implementation as a high-risk stratification criterion in frontline AIEOP-BFM trials for the treatment of ALL.

Despite these promises, there are limitations with our approach. First, besides polymerase chain reaction analysis for *ERG* deletions, our study used MLPA-based assessment of leukemic copy number aberrations. This assay has a limited sensitivity to detect less-abundant subclonal lesions (< 30%). However, the prognostic significance of these subclonal lesions is unclear, and the relevance of their exact quantification currently is a matter of debate.[49,53] Second, although we initiated our analysis with 991 patients, we may have missed additional prognostically relevant deletions as a result of a lack of power. In this context, continued analyses, including the pooling of copy number variation data across study groups, will secure identification of additional combinations with prognostic effect. Third, in our simple approach, we only incorporated the data available to us, which led to a restricted view by potentially excluding additional relevant information. The capture of broader information by more-comprehensive genomic techniques could further expand and strengthen the *IKZF1*^{plus} profile. Fourth, we lacked information on other relevant prognostic subgroups. For example, *IKZF1* deletions frequently are found in Philadelphia-like and *BCR-ABL1*–like ALL, two related subgroups characterized by tyrosine kinases or cytokine receptor activation. Indeed, Roberts et al[54] described a negative prognostic effect for *IKZF1* deletions in Philadelphia-like ALL. In contrast, a similar effect could not be observed by van der Veer et al[48] when analyzing the role of *IKZF1* deletions in *BCR-ABL1*–like ALL. Finally, because of a lack of systematic assessment, we were not able to compare our approach with other existing ones that integrated *IKZF1* deletion status with molecular and/or conventional diagnostic genetic information with or without MRD data.[33,49,50] For example, we did not routinely analyze *IGH-CRLF2* fusions in our cohort and, therefore, cannot judge their possible prognostic significance alone or in the context of *IKZF1* deletion. Future, more-comprehensive prospective studies will need to determine the potential interactions of *IKZF1*^{plus} with kinase-activating and other poor prognosis genetic aberrations in our trials.

One intriguing observation related to *IKZF1*^{plus} is the strong dependence of its prognostic effect on MRD. In MRD-SR patients with no measurable MRD after induction, treatment outcome was not negatively affected by the presence of *IKZF1*^{plus}, whereas patients with measureable MRD (MRD-IR, MRD-HR) faced a 10-fold higher relapse rate. Previous studies, including ours, described an association of *IKZF1* deletion and MRD in ALL. However, the effects observed in these studies were not comparable with regard to the dramatic extent observed in the current study,[27,33,52] or studies were performed under different conditions with regard to MRD assessment, risk stratification, or treatment.[46,51,55] Hypothetical explanations for the effect observed in the current study could be different distributions of either additional leukemic aberrations or underlying hereditary factors in MRD-SR compared with MRD-IR and MRD-HR patients. Future research efforts will need to discover the mechanism that underlies the differential prognostic effect of *IKZF1*^{plus} in various MRD risk groups.

With speculation on the characteristics that unify *IKZF1*^{plus} patients as a subgroup, the ALL risk-conferring *GATA3* single-nucleotide variant rs3824662[45,46] was found to be enriched within *IKZF1*^{plus} patients. This may point to a common hereditary background in these patients, which needs additional characterization.

In conclusion, we have integrated molecular genetic data with MRD data into a single combined classification that will be used to refine treatment stratification and guide innovative but still costly therapeutic applications in our upcoming trial AIEOP-BFM ALL 2017 for frontline treatment of pediatric ALL. To go beyond current risk stratification strategies, additional research on the biologic basis of our observations is needed and will be helpful in guiding the development of more-targeted and less-toxic innovative therapies for children and adolescents with ALL.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Martin Stanulla, Martina U. Muckenthaler, Gunnar Cario, Martin Schrappe, Martin Zimmermann
Financial support: Martin Stanulla, Andrea Biondi, Giuseppe Basso, Renate Panzer-Grümayer, Martin Schrappe
Administrative support: Andrea Biondi, Giuseppe Basso, Renate Panzer-Grümayer, Martin Schrappe, Martin Stanulla
Provision of study materials or patients: Andrea Biondi, Giuseppe Basso, Gunnar Cario, Cornelia Eckert, Richard S. Houlston, Renate Panzer-Grümayer, Martin Schrappe, Martin Stanulla
Collection and assembly of data: Martin Stanulla, Elif Dagdan, Marketa Zaliova, Anja Möricke, Chiara Palmi, Giovanni Cazzaniga, Cornelia Eckert, Geertruy te Kronnie, Rolf Koehler, Claus R. Bartram, Wolf-Dieter Ludwig, Kirsten Bleckmann, Stefanie Groeneveld-Krentz, Denis Schewe, Andrea Biondi, Andreas Kulozik, Giuseppe Basso, Maria Grazia Valsecchi, Britt-Sabrina Petersen, Andre Franke, Petra Dörge, Doris Steinemann, Oskar A. Haas, Renate Panzer-Grümayer, Hélène Cavé, Richard S. Houlston
Data analysis and interpretation: Martin Stanulla, Elif Dagdan, Marketa Zaliova, Anja Möricke, Giovanni Cazzaniga, Cornelia Eckert, Geertruy te Kronnie, Jean-Pierre Bourquin, Beat Bornhauser, Stefanie V. Junk, Laura Hinze, Norman Klein, Christian P. Kratz, Andrea Biondi, Arndt Borkhardt, Andreas Kulozik, Giuseppe Basso, Maria Grazia Valsecchi, Shai Izraeli, Renate Panzer-Grümayer, Hélène Cavé, Gunnar Cario, Martin Schrappe, Martin Zimmermann
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Affiliations

Martin Stanulla, **Elif Dagdan**, **Stefanie V. Junk**, **Laura Hinze**, **Norman Klein**, **Christian P. Kratz**, **Petra Dörge**, **Doris Steinemann**, and **Martin Zimmermann**, Hannover Medical School; **Petra Dörge**, German Center for Infection Research, Hannover; **Anja Möricke**, **Kirsten Bleckmann**, **Denis Schewe**, **Gunnar Cario**, and **Martin Schrappe**, University Hospital Schleswig-Holstein; **Britt-Sabina Petersen** and **Andre Franke**, Kiel University, Kiel; **Cornelia Eckert** and **Stefanie Groeneveld-Krentz**, Charité University Hospital; **Wolf-Dieter Ludwig**, HELIOS-Clinic Berlin-Buch, Berlin; **Rolf Koehler**, **Claus R. Bartram**, **Andreas Kulozik**, and **Martina U. Muckenthaler**, University of Heidelberg, Heidelberg; **Arndt Borkhardt**, Heinrich-Heine University, Düsseldorf, Germany; **Marketa Zaliova**, Charles University and University Hospital Motol, Prague, Czech Republic; **Chiara Palmi**, **Giovanni Cazzaniga**, and **Andrea Biondi**, Azienda Ospedaliera San Gerardo; **Maria Grazia Valsecchi**, University of Milano-Bicocca, Monza; **Geertruy te Kronnie** and **Giuseppe Basso**, University of Padova, Padua, Italy; **Jean-Pierre Bourquin** and **Beat Bornhauser**, University Children's Hospital Zurich, Zurich, Switzerland; **Shai Izraeli**, Sheba Medical Center Tel-Hashomer and Tel Aviv University, Tel Aviv, Israel; **Oskar A. Haas** and **Renate Panzer-Grümayer**, St Anna Kinderkrebsforschung and Medical University Vienna, Vienna, Austria; **Hélène Cavé**, Robert Debré Hospital and Paris-Diderot University, Paris, France; and **Richard S. Houlston**, The Institute of Cancer Research, London, United Kingdom.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

IKZF1[plus] Defines a New Minimal Residual Disease–Dependent Very-Poor Prognostic Profile in Pediatric B-Cell Precursor Acute Lymphoblastic Leukemia

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Martin Stanulla
Honoraria: Baxalta

Elif Dagdan
No relationship to disclose

Marketa Zaliova
No relationship to disclose

Anja Möricke
Honoraria: Baxalta, Jazz Pharmaceuticals
Travel, Accommodations, Expenses: Jazz Pharmaceuticals

Chiara Palmi
No relationship to disclose

Giovanni Cazzaniga
No relationship to disclose

Cornelia Eckert
No relationship to disclose

Geertruy te Kronnie
No relationship to disclose

Jean-Pierre Bourquin
Honoraria: Amgen

Beat Bornhauser
No relationship to disclose

Rolf Koehler
No relationship to disclose

Claus R. Bartram
No relationship to disclose

Wolf-Dieter Ludwig
No relationship to disclose

Kirsten Bleckmann
Travel, Accommodations, Expenses: Jazz Pharmaceuticals

Stefanie Groeneveld-Krentz
No relationship to disclose

Denis Schewe
No relationship to disclose

Stefanie V. Junk
No relationship to disclose

Laura Hinze
No relationship to disclose

Norman Klein
No relationship to disclose

Christian P. Kratz
No relationship to disclose

Andrea Biondi
No relationship to disclose

Arndt Borkhardt
No relationship to disclose

Andreas Kulozik
No relationship to disclose

Martina U. Muckenthaler
Honoraria: Novartis, Silence Therapeutics
Consulting or Advisory Role: Merck
Research Funding: Novartis, Silence Therapeutics

Giuseppe Basso
No relationship to disclose

Maria Grazia Valsecchi
No relationship to disclose

Shai Izraeli
Speakers' Bureau: prIME Oncology

Britt-Sabrina Petersen
No relationship to disclose

Andre Franke
No relationship to disclose

Petra Dörge
No relationship to disclose

Doris Steinemann
No relationship to disclose

Oskar A. Haas
No relationship to disclose

Renate Panzer-Grümayer
No relationship to disclose

Hélène Cavé
Honoraria: Bristol-Myers Squibb
Consulting or Advisory Role: Roche

Richard S. Houlston
No relationship to disclose

Gunnar Cario
No relationship to disclose

Martin Schrappe
Honoraria: prIME Oncology
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Martin Zimmermann
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Appendix

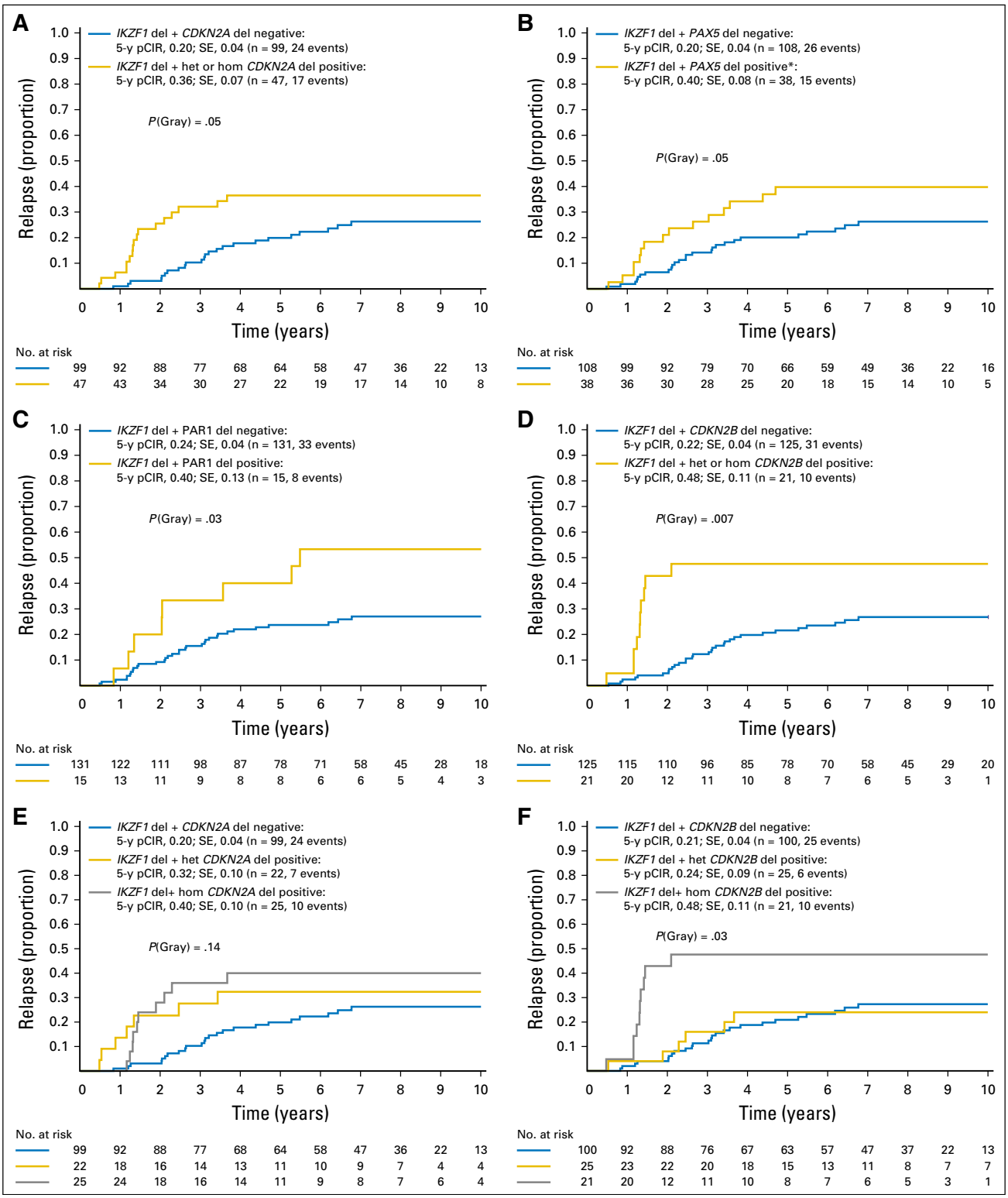

Fig A1. Cumulative relapse incidence (CIR) at five years in B-cell precursor ALL patients treated on trial AIEOP-BFM (Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) ALL 2000 according to *IKZF1* status alone or in combination with (A) heterozygous or homozygous *CDKN2A* deletion, (B) heterozygous or homozygous *PAX5* deletion, (C) PAR1 deletion, (D) homozygous *CDKN2B* deletion. (E) and (F) show *CDKN2A* and *CDKN2B* wild-type, heterozygous and homozygous deletions separately. (*) Fourteen patients with heterozygous (het) *PAX5* deletion, one patient with homozygous (hom) *PAX5* deletion (del). pCIR, estimated cumulative incidence of relapse.

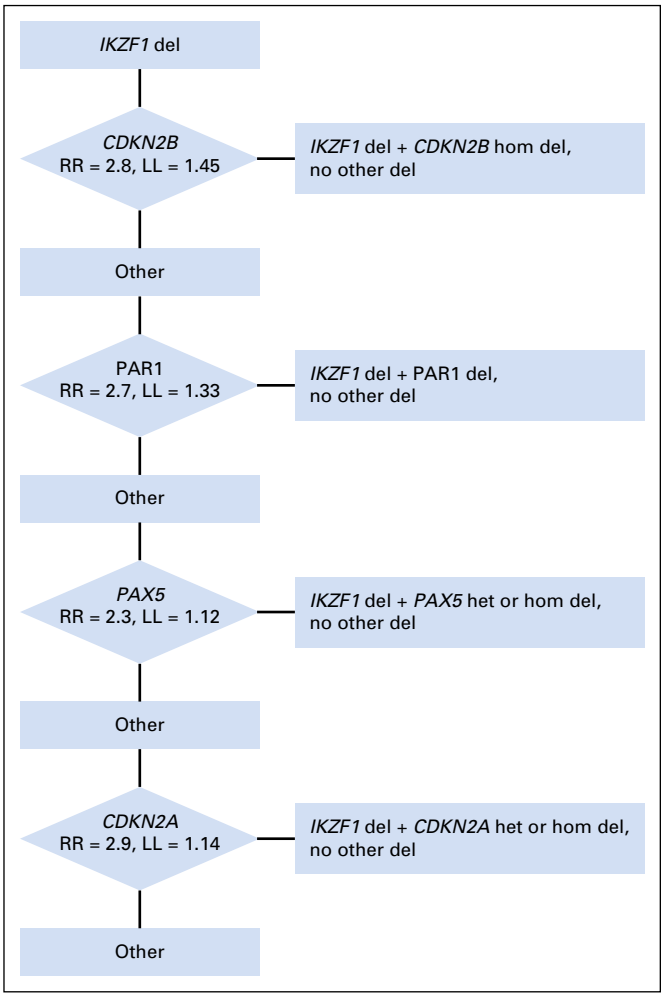

Fig A2. Classification and regression tree analysis of sequentially subclassified patients by genetic data from multiplex ligation-dependent probe amplification analysis that included *PAX5*, *ETV6*, *RB1*, *BTG1*, *EBF1*, *CDKN2A*, *CDKN2B*, or PAR1 for the 146 patients with *IKZF1* deletion (del) from AIEOP-BFM (Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) Acute Lymphoblastic Leukemia (ALL) 2000 for whom these data were available. The most discriminatory variable at each point is selected by the classification and regression tree algorithm. The groups were defined by the observed incidence of relapse within each node of the graph for deleted and nondeleted cases, respectively. Relapse risk ratio (RR) and lower-level CIs (LL) are presented. het, heterozygous; hom, homozygous.

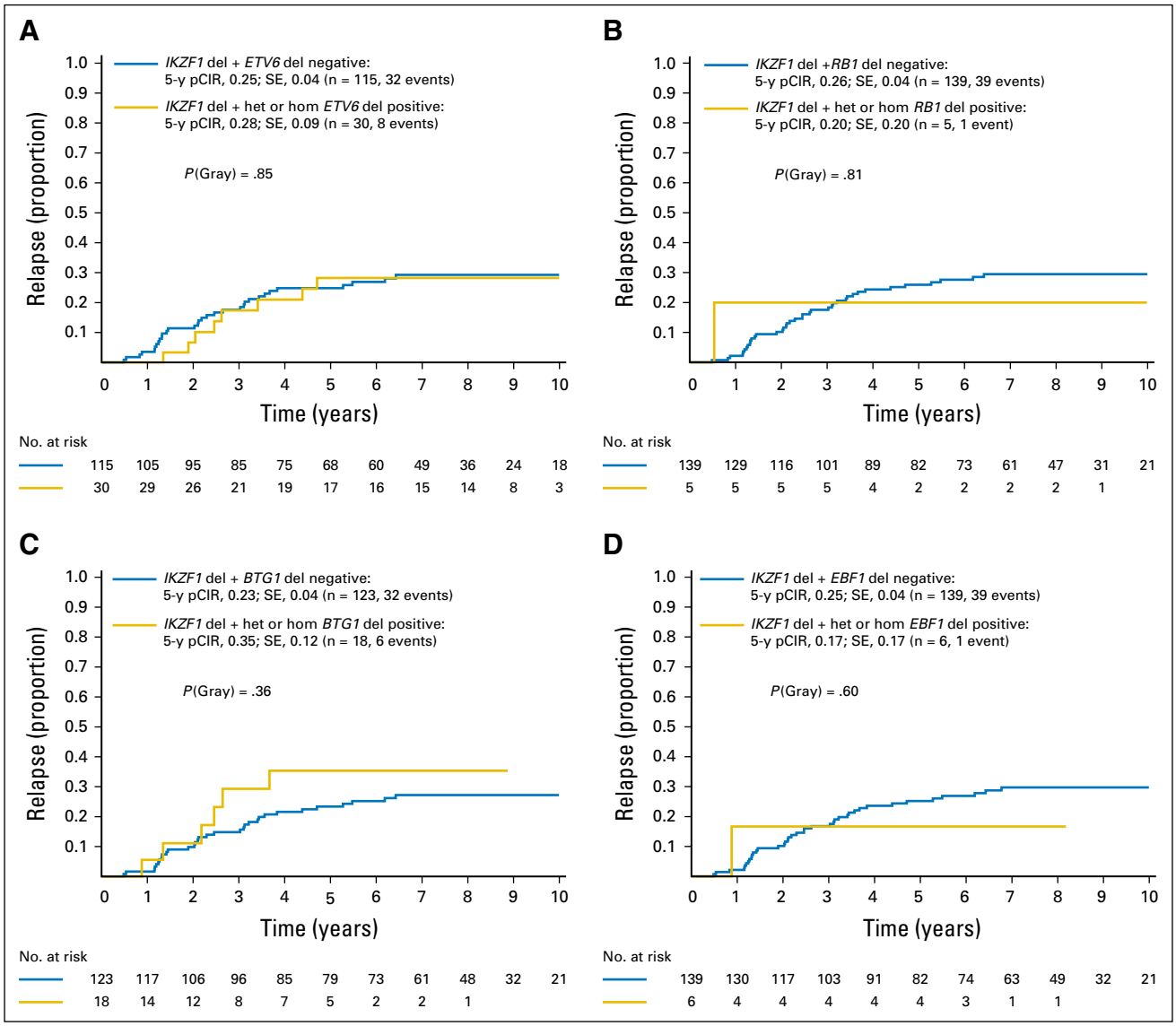

Fig A3. Cumulative relapse incidence (CIR) at five years in B-cell precursor ALL patients treated on trial AIEOP-BFM (Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) ALL 2000 according to *IKZF1* status alone or in combination with (A) heterozygous or homozygous *ETV6* deletion, (B) heterozygous or homozygous *RB1* deletion, (C) heterozygous or homozygous *BTG1* deletion, (D) heterozygous or homozygous *EBF1* deletion. pCIR, estimated cumulative incidence of relapse. del, deletion; het, heterozygous; hom, homozygous.

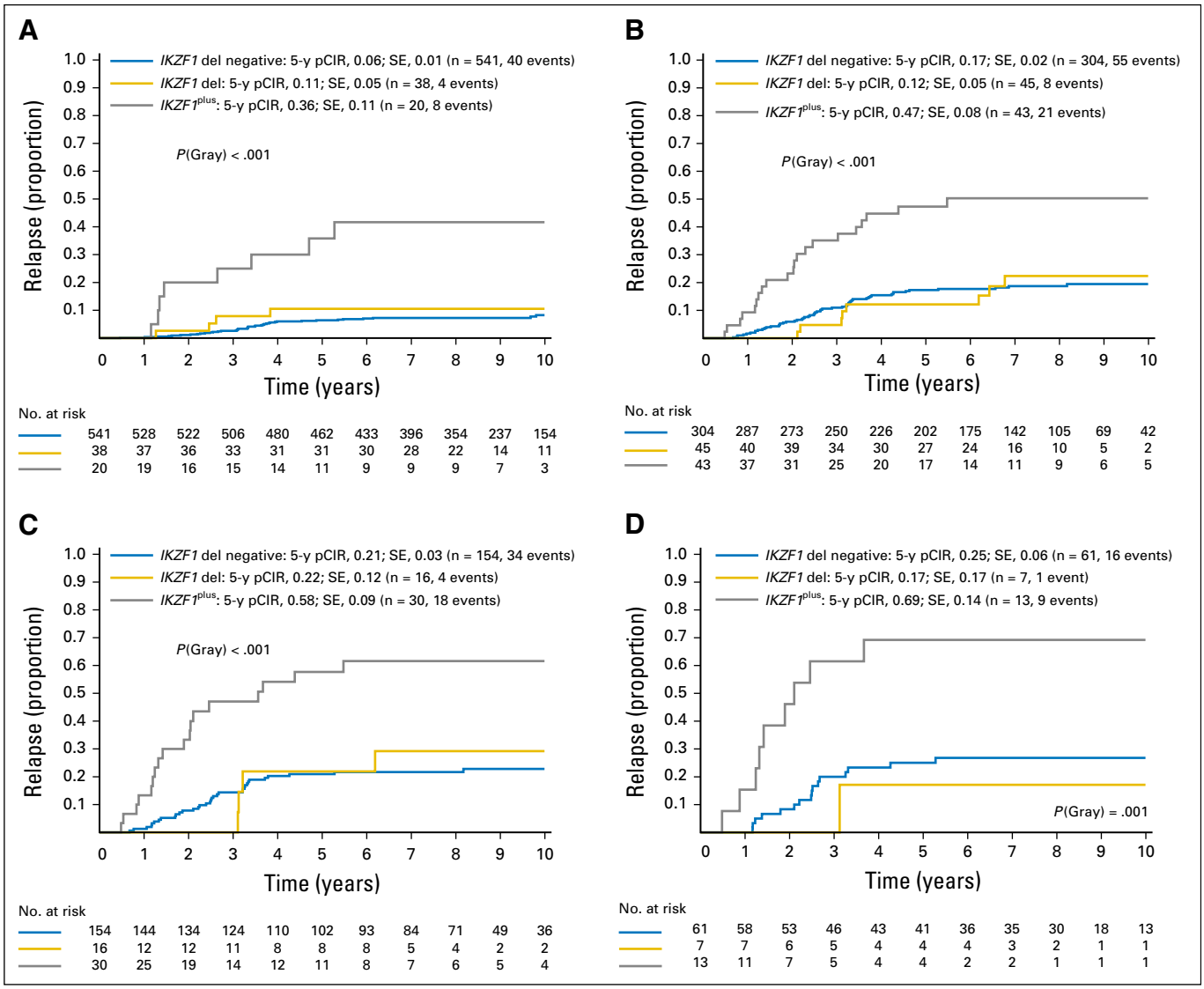

Fig A4. Cumulative relapse incidence (CIR) at five years in B-cell precursor ALL patients treated on trial AIEOP-BFM (Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) ALL 2000 stratified by white blood cell (WBC) count and NCI risk groups according to *IKZF1* status (no *IKZF1* deletion, *IKZF1*-deleted, *IKZF1*^{plus}). NCI standard-risk patients were one to younger than ten years and had an initial WBC count of $< 50{,}000/\mu l$, NCI high-risk patients were ten years and older and/or had an initial WBC of $\geq 50{,}000/\mu l$. CIR in (A) NCI standard-risk patients, (B) NCI high-risk patients, (C) patients with initial WBC counts $\geq 50{,}000/\mu l$, and (D) patients with initial WBC counts of $\geq 100{,}000/\mu l$. pCIR, estimated cumulative incidence of relapse. del, deletion.

Table A1. Deletion Matrix for 991 German Patients With B-Cell Precursor ALL Treated in Trial AIEOP-BFM ALL 2000

	No *IKZF1* del		*IKZF1* del†		
Deletion Combination*	*ERG* del Negative	*ERG* del Positive	*ERG* del Negative	*ERG* del Positive	*IKZF1*^{plus}‡
CDKN2A wild type					
PAX5 wild type					
CDKN2B wild type					
PAR1 wild type	516	22	57	19	
PAR1 del	4	—	—		9
CDKN2B het del					
PAR1 wild type	5	—	1	1	
CDKN2B hom del					
PAR1 wild type	2	—			
PAX5 het or hom del					
CDKN2B wild type					
PAR1 wild type	65	3		1	9
PAR1 del	3	—			2
CDKN2B hom del					
PAR1 wild type	1	—			
CDKN2A het del					
PAX5 wild type					
CDKN2B wild type					
PAR1 wild type	12	1			2
PAR1 del	1	—			
CDKN2B het del					
PAR1 wild type	53	2		1	5
PAR1 del	1	—			
CDKN2B hom del					
PAR1 wild type	8	—			2
PAR1 del	1	—			
PAX5 het or hom del					
CDKN2B wild type					
PAR1 wild type	2	—			1
CDKN2B het del					
PAR1 wild type	22	3		2	4
PAR1 del	1	—			2
CDKN2B hom del					
PAR1 wild type	8	—			2
PAR1 del	2	—			1
CDKN2A hom del					
PAX5 wild type					
CDKN2B wild type					
PAR1 wild type	1	—			
PAR1 del	1	—			
CDKN2B het del					
PAR1 wild type	6	1			3
PAR1 del	—	—			1
CDKN2B hom del					
PAR1 wild type	39	1			7
PAR1 del	3	—			
PAX5 het or hom del					
CDKN2B wild type					
PAR1 wild type	2	—			
CDKN2B het del					
PAR1 wild type	10	—			5
PAR1 del	2	—			
CDKN2B hom del					
PAR1 wild type	38	—		1	8
PAR1 del	3	—			

Abbreviations: AIEOP-BFM, Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; del, deletion; het, heterozygous; hom, homozygous.

*On the basis of multiplex ligation-dependent probe amplification analysis for *IKZF1*, *CDKN2A*, *PAX5*, *CDKN2B*, and PAR1 according to genomic polymerase chain reaction for *ERG*.

†*IKZF1* del present but does not fulfill the *IKZF1*^{plus} definition.

‡Presence of *IKZF1* del and at least an additional deletion in *PAX5*, *CDKN2A*, *CDKN2B*, or PAR1 in the absence of *ERG* del.

Table A2. Clinical Characteristics According to *IKZF1* Status in 417 Italian Patients With Precursor B-Cell ALL Treated in Trial AIEOP-BFM ALL 2000

Characteristic	*IKZF1* Status, No. (%)			*P**
	No *IKZF1* Deletion	*IKZF1* Deletion	*IKZF1*^{plus}	
No. of patients	357	42	18	
Sex				
Male	188 (52.7)	22 (52.4)	8 (44.4)	
Female	169 (47.3)	20 (47.6)	10 (55.6)	.793
Age at diagnosis, years				
< 10	307 (86.0)	28 (66.7)	12 (66.7)	
≥ 10	50 (14.0)	14 (33.3)	6 (33.3)	.001
Presenting WBC, count/μL				
< 10,000	199 (55.7)	18 (42.9)	5 (27.8)	
10,000 to < 50,000	111 (31.1)	18 (42.9)	5 (27.8)	
50,000 to < 100,000	23 (6.4)	2 (4.8)	5 (27.8)	
≥ 100,000	24 (6.7)	4 (9.5)	3 (16.7)	< .001
CNS disease†				
Negative	357 (100)	42 (100)	18 (100)	—
Positive	—	—	—	
Other/unknown	—	—	—	
Hyperdiploidy‡				
No	251 (70.3)	33 (78.6)	17 (94.4)	
Yes	81 (22.7)	5 (11.9)	—	.023
Unknown	25 (7.0)	4 (9.5)	1 (5.6)	
ETV6-RUNX1				
Negative	257 (72.0)	38 (90.5)	17 (94.4)	
Positive	82 (23.0)	—	1 (5.6)	< .001
Unknown	18 (5.0)	4 (9.5)	—	
MLL-AF4				
Negative	352 (98.6)	42 (100)	18 (100)	
Positive	3 (0.8)	—	—	.775
Unknown	2 (0.6)	—	—	
Prednisone response§				
Good	333 (93.3)	39 (92.9)	17 (94.4)	
Poor	21 (5.9)	3 (7.1)	—	.550
Unknown	3 (0.8)	—	1 (5.6)	
MRD‖				
TP1 and TP2 negative	116 (32.5)	5 (11.9)	3 (16.7)	
Other	162 (45.4)	20 (47.6)	8 (44.4)	
TP2 ≥ 10^{-3}	3 (0.8)	6 (14.3)	—	< .001
Unknown	76 (21.3)	11 (26.2)	7 (38.9)	
Final risk group¶				
SR	109 (30.5)	5 (11.9)	3 (16.7)	
IR	222 (62.2)	30 (71.4)	12 (66.7)	
HR	26 (7.3)	7 (16.7)	3 (16.7)	< .001

NOTE. *IKZF1*^{plus} definition: presence of *IKZF1* deletion and at least an additional deletion in *PAX5*, *CDKN2A*, *CDKN2B*, or PAR1 in the absence of *ERG* deletion. *IKZF1* deletion definition: *IKZF1* deletion present but does not fulfill the *IKZF1*^{plus} definition.

Abbreviations: AIEOP-BFM, Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; HR, high risk; IR, intermediate risk; MRD, minimal residual disease; SR, standard risk; TP, time point.

*χ^2 or Fisher's exact test that compares the groups with various *IKZF1* statuses.

†CNS negative, puncture nontraumatic without leukemic blasts in the CSF after cytocentrifugation; CNS positive, puncture nontraumatic with WBC > 5/μL in the CSF with identifiable blasts.

‡Defined by cytogenetics (> 50 chromosomes) or by flow cytometric analyses of the ratio of DNA content of leukemic G0/G1 cells to normal diploid lymphocytes (≥ 1.16).

§Good, < 1,000 leukemic blood blasts/μL on treatment day 8; poor, ≥ 1,000/μL.

‖TP1, treatment day 33; TP2, treatment day 78; MRD ≥ 10^{-3} qualifies for the MRD-HR group.

¶For definition of risks, see Patients and Methods, under Sample Selection.

Table A3. Estimated HRs From the Multivariable Cox Proportional Hazards Model on Event-Free Survival and Hazard of Relapse in Patients With B-Cell Precursor ALL in Trial AIEOP-BFM ALL 2000

Variable	Event			Relapse		
	HR	95% CI	*P*	HR	95% CI	*P*
IKZF1^{plus}*	2.95	1.02 to 8.55	.046	3.16	1.08 to 9.28	.036
IKZF1 deletion†	0.85	0.38 to 1.88	.688	0.84	0.37 to 1.90	.681
ETV6-RUNX1 positivity‡	2.26	0.95 to 5.34	.064	2.12	0.89 to 5.05	.088
MRD standard risk§	0.34	0.16 to 0.71	.004	0.36	0.17 to 0.76	.004
MRD high risk§	23.49	6.82 to 80.85	< .001	33.53	9.67 to 116.30	.018
Slow early MRD response‖	2.08	1.08 to 4.02	.029	2.22	1.14 to 4.31	.018
Poor prednisone response¶	0.40	0.10 to 1.56	.186	0.29	0.06 to 1.31	.107
Presenting WBC# count ≥ 100,000/μL	1.18	0.40 to 3.43	.767	1.07	0.34 to 3.36	.907

Abbreviations: AIEOP-BFM, Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; HR, hazard ratio; MRD, minimal residual disease.

*Presence of *IKZF1* deletion and at least an additional deletion in *PAX5*, *CDKN2A*, *CDKN2B*, or PAR1 in the absence of *ERG* deletion. HR compared with patients who lacked an *IKZF1* deletion.

†*IKZF1* deletion present but does not fulfill the *IKZF1*^{plus} definition. HR compared with patients who lacked an *IKZF1* deletion.

‡HR compared with *ETV6-RUNX1* negative.

§MRD standard risk, negative on treatment days 33 and 78; MRD high risk, leukemic cell load ≥ 5×10^{-4} on treatment day 78; all other results MRD intermediate risk. HR compared with other MRD groups.

‖MRD ≥ 5×10^{-4} on treatment day 33 and positivity of < 5×10^{-4} on treatment day 78. HR compared with MRD intermediate risk patients with no slow early response.

¶Leukemic blasts ≥ 1,000/μL in the peripheral blood on treatment day 8. HR compared with patients with < 1,000/μL leukemic blasts.

#HR compared with patients with presenting WBC counts < 100,000/μL.

Table A4. Deletions in *IKZF1*^{plus} Patients in Trial AIEOP-BFM ALL 2000 by Various MRD Groups

Type of Deletion	MRD Group*, No. (%)			
	SR	IR	HR	Unknown
No. of patients	19	27	10	7
CDKN2A wild type	5 (26.3)	9 (33.3)	3 (30.0)	3 (42.9)
CDKN2A het or hom del	14 (73.7)	18 (66.6)	7 (70.0)	4 (57.1)
PAX5 wild type	6 (31.6)	11 (40.7)	7 (70.0)	5 (71.4)
PAX5 het or hom del	13 (68.4)	16 (59.3)	3 (30.0)	2 (28.6)
CDKN2B wild type or het del	12 (63.2)	19 (70.4)	6 (60.0)	6 (85.7)
CDKN2B hom del	7 (36.8)	8 (29.6)	4 (40.0)	1 (14.3)
PAR1 wild type	15 (78.9)	20 (74.1)	9 (90.0)	5 (71.4)
PAR1 del	4 (21.1)	7 (25.9)	2 (20.0)	2 (28.6)

NOTE. On the basis of a multiplex ligation-dependent probe amplification analysis. *IKZF1*^{plus} definition: presence of *IKZF1* deletion and at least an additional deletion in *PAX5* (het or hom), *CDKN2A* (het or hom), *CDKN2B* (hom only), or PAR1 (het or hom) in the absence of *ERG* deletion (het or hom).

Abbreviations: AIEOP-BFM, Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; del, deletion; het, heterozygous; hom, homozygous; HR, high risk; IR, intermediate risk; MRD, minimal residual disease; SR, standard risk.

*MRD-SR, negative on treatment days 33 and 78; MRD-HR, leukemic cell load ≥ 5×10^{-4} on treatment day 78; all other results MRD-IR.

Table A5. Number of Unfavorable Deletions in Addition to *IKZF1* in *IKZF1*^{plus} Patients in Trial AIEOP-BFM ALL 2000 by Various MRD Groups

	MRD Group*, No. (%)			
No. of deletions	SR	IR	HR	Unknown
No. of patients	19	27	10	7
One deletion	6 (31.6)	12 (44.4)	5 (50.0)	5 (71.4)
Two deletions	7 (36.8)	9 (33.3)	4 (40.0)	2 (28.6)
Three deletions	6 (31.6)	5 (18.5)	1 (10.0)	—
Four deletions	—	1 (3.7)	—	—

NOTE. On the basis of a multiplex ligation-dependent probe amplification analysis, including *CDKN2A*, *PAX5*, *CDKN2B*, and PAR1. *IKZF1*^{plus} definition: presence of *IKZF1* deletion and at least an additional deletion in *PAX5* (het or hom), *CDKN2A* (het or hom), *CDKN2B* (hom only), or PAR1 (het or hom) in the absence of *ERG* deletion (het or hom).
Abbreviations: AIEOP-BFM, Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; het, heterozygous; hom, homozygous; HR, high risk; IR, intermediate risk; MRD, minimal residual disease; SR, standard risk.
*MRD-SR, negative on treatment days 33 and 78; MRD-HR, leukemic cell load $\geq 5 \times 10^{-4}$ on treatment day 78; all other results MRD-IR. $P = .750$ by χ^2 test for comparison of the various MRD groups for differences in number of deletions.

Table A6. Genes Affected in Deletion Patterns at Initial Diagnosis and at Relapse in 14 *IKZF1*^{plus} Patients

Sample Pair	Initial Diagnosis	Relapse Diagnosis
1	*IKZF1* het, *PAX5* het	*IKZF1* het, *CDKN2A* het, *CDKN2B* het, *PAX5* het
2	*IKZF1* het, *CDKN2A* hom, *CDKN2B* hom, *PAX5* het	*IKZF1* het, *CDKN2A* het, *CDKN2B* het
3	*IKZF1* het, *PAX5* het	*IKZF1* het, *PAX5* het
4	*IKZF1* het, PAR1 het	*IKZF1* het
5	*IKZF1* het, PAR1 het	*IKZF1* het
6	*IKZF1* het, *PAX5* het	*IKZF1* het, *CDKN2A* het, *CDKN2B* hom, *PAX5* hom
7	*IKZF1* het, *CDKN2A* hom, *CDKN2B* het, *PAX5* het	*IKZF1* het, *CDKN2A* hom, *CDKN2B* het, *PAX5* het
8	*IKZF1* hom, *CDKN2A* hom, *CDKN2B* hom	*IKZF1* hom, *CDKN2A* hom, *CDKN2B* hom
9	*IKZF1* het, *PAX5* het	*IKZF1* het, *PAX5* het
10	*IKZF1* het, PAR1 het	*IKZF1* het, PAR1 het
11	*IKZF1* het, *CDKN2A* het, *CDKN2B* hom, *PAX5* het	*IKZF1* het, *CDKN2A* het, *CDKN2B* hom, *PAX5* het
12	*IKZF1* het, *CDKN2A* hom, *CDKN2B* het	*IKZF1* het, *CDKN2A* het, *CDKN2B* het
13	*IKZF1* het, *CDKN2A* het, *CDKN2B* hom, *PAX5* hom, PAR1 het	*IKZF1* het, *CDKN2A* het, *CDKN2B* hom, *PAX5* hom, PAR1 het
14	*IKZF1* het, *PAX5* het	*IKZF1* het, *PAX5* het

NOTE. On the basis of a multiplex ligation-dependent probe amplification analysis, including *CDKN2A*, *PAX5*, *CDKN2B*, and PAR1. *IKZF1*^{plus} definition: presence of *IKZF1* deletion and at least an additional deletion in *PAX5* (het or hom), *CDKN2A* (het or hom), *CDKN2B* (hom only), or PAR1 (het or hom) in the absence of *ERG* deletion (het or hom).
Abbreviations: het, heterozygous; hom, homozygous.