ORIGINAL ARTICLE

Deletion of *IKZF1* and Prognosis in Acute Lymphoblastic Leukemia

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ABSTRACT

BACKGROUND

Despite best current therapy, up to 20% of pediatric patients with acute lymphoblastic leukemia (ALL) have a relapse. Recent genomewide analyses have identified a high frequency of DNA copy-number abnormalities in ALL, but the prognostic implications of these abnormalities have not been defined.

METHODS

We studied a cohort of 221 children with high-risk B-cell–progenitor ALL with the use of single-nucleotide–polymorphism microarrays, transcriptional profiling, and resequencing of samples obtained at diagnosis. Children with known very-high-risk ALL subtypes (i.e., *BCR-ABL1*–positive ALL, hypodiploid ALL, and ALL in infants) were excluded from this cohort. A copy-number abnormality was identified as a predictor of poor outcome, and it was then tested in an independent validation cohort of 258 patients with B-cell–progenitor ALL.

RESULTS

More than 50 recurring copy-number abnormalities were identified, most commonly involving genes that encode regulators of B-cell development (in 66.8% of patients in the original cohort); *PAX5* was involved in 31.7% and *IKZF1* in 28.6% of patients. Using copy-number abnormalities, we identified a predictor of poor outcome that was validated in the independent validation cohort. This predictor was strongly associated with alteration of *IKZF1*, a gene that encodes the lymphoid transcription factor IKAROS. The gene-expression signature of the group of patients with a poor outcome revealed increased expression of hematopoietic stem-cell genes and reduced expression of B-cell–lineage genes, and it was similar to the signature of *BCR-ABL1*–positive ALL, another high-risk subtype of ALL with a high frequency of *IKZF1* deletion.

CONCLUSIONS

Genetic alteration of *IKZF1* is associated with a very poor outcome in B-cell–progenitor ALL.

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Cure rates among children with acute lymphoblastic leukemia (ALL) now exceed 80%,[1] but current therapies have substantial toxic effects, and up to 20% of patients with ALL have a relapse after initial therapy.[2] Risk stratification in B-cell–progenitor ALL is based on a number of recurring chromosomal abnormalities, including hyperdiploidy, hypodiploidy, translocations t(12;21)(*ETV6-RUNX1*), t(9;22)(*BCR-ABL1*), and t(1;19)(*TCF3-PBX1*), and rearrangement of the mixed-lineage leukemia (*MLL*) gene. Treatment failure is common in *BCR-ABL1*–rearranged and *MLL*-rearranged ALL, but relapse occurs in all subtypes. Moreover, the biologic basis of resistance to therapy in ALL is poorly understood.

Recent genomewide analyses of DNA copy-number abnormalities have identified numerous recurring genetic alterations in ALL.[3-6] Mutations of genes encoding transcriptional regulators of B lymphoid development, including *PAX5*, *EBF1*, and *IKZF1*, occur in more than 40% of patients with B-cell–progenitor ALL.[3] Deletion of *IKZF1*, which encodes the lymphoid transcription factor IKAROS, is a very frequent event in *BCR-ABL1*–positive ALL and at the progression of chronic myeloid leukemia to lymphoid blast crisis.[5] Other copy-number abnormalities involve tumor suppressors and cell-cycle regulators (e.g., *CDKN2A/B*, *RB1*, *PTEN*, and *ETV6*), regulators of apoptosis (*BTG1*), drug-receptor genes (*NR3C1* and *NR3C2*), and lymphoid-signaling molecules (*BTLA* and *CD200*).[3]

We report on a study of copy-number abnormalities in 221 children with high-risk ALL. We identified a predictor of poor outcome based on copy-number abnormalities that was driven by the deletion or mutation of *IKZF1*, which is associated with a high risk of relapse. The correlation between this predictor and poor outcome was validated in an independent cohort of 258 patients with B-cell–progenitor ALL. The predictor was also associated with a gene-expression signature characterized by the increased expression of hematopoietic stem-cell genes and the reduced expression of B lymphoid genes.

METHODS

PATIENTS AND SAMPLES

Of the two cohorts of patients who were examined (see the Supplementary Appendix, available with the full text of this article at NEJM.org), the original cohort comprised 221 patients with B-cell–progenitor ALL treated in the Children's Oncology Group P9906 study; this study used an augmented reinduction–reconsolidation strategy (the Berlin–Frankfurt–Münster regimen) (Table 1 in the Supplementary Appendix).[7,8] All patients were at high risk for treatment failure based on the presence of central nervous system or testicular disease, *MLL* gene rearrangement, or age, sex, and leukocyte count at presentation.[9] Patients with *BCR-ABL1*–positive and hypodiploid ALL, infants, and patients who did not have a response to induction chemotherapy were excluded. A recurring chromosomal abnormality was not detected in 170 patients (76.9%). Patients were enrolled from May 2000 through April 2003. The median follow-up time, defined as the time from enrollment to death or the last follow-up, was 3.94 years (range, 0.16 to 6.20).

The validation cohort comprised 258 children with B-cell–progenitor ALL treated in multiple protocols[3,5,10-14] at St. Jude Children's Research Hospital. This cohort included both standard-risk and high-risk patients, patients with common aneuploidies, and patients with recurring translocations (including 21 *BCR-ABL1*–positive patients) (Table 2 in the Supplementary Appendix). Patients were enrolled from September 1986 through February 2007. The median follow-up time was 6.05 years (range, 0.27 to 21.47).

Written informed consent and institutional-review-board approval were obtained for both cohorts. In the original cohort, minimal residual disease was measured in 196 patients at day 8 (in peripheral blood) and in 204 patients at day 29 (in bone marrow) of initial induction chemotherapy, and in the validation cohort, in 161 patients at day 19 and in 160 patients at day 46. This measurement of minimal residual disease was performed with the use of immunophenotyping, as previously described.[8,15,16]

No commercial entity was involved in the conduct of the study, the analysis or storage of the data, or the preparation of the manuscript. The authors vouch for the completeness and accuracy of the data and the analysis.

GENOMIC ANALYSES

DNA extracted from leukemic cells obtained at diagnosis and from samples obtained during remission was genotyped with the use of 250k Sty and Nsp single-nucleotide–polymorphism (SNP)

arrays (Affymetrix). Samples from patients in the validation cohort were genotyped with SNP 6.0 arrays in 36 patients, 250K Sty and Nsp arrays in 37 patients, and 250K Sty and Nsp and 50K Hind 240 and Xba 240 arrays in 185 patients. SNP array analyses, gene-expression profiling, and the use of gene set enrichment analysis[17] and gene set analysis[18] to compare gene-expression signatures and examine associations between gene sets and outcome are described in the Supplementary Appendix.

GENOMIC RESEQUENCING OF *PAX5*, *EBF1*, AND *IKZF1*

Genomic resequencing of all the coding exons of *PAX5*, *EBF1*, and *IKZF1* was performed for all samples in the original cohort. The Supplementary Appendix includes a description of sequencing methods and structural modeling of *PAX5* mutations.

DNA COPY-NUMBER ABNORMALITIES AND OUTCOME

Supervised principal-components analysis[19,20] was used to examine associations between copy-number abnormalities and treatment outcome in a genomewide fashion (Supplementary Appendix). A modified univariate Cox score was calculated for the association between the copy-number status of each gene and the cumulative risk of any adverse events or relapse, and genes with a Cox score that exceeded a threshold that best predicted outcome were used to perform a principal-components analysis. We subsequently generated a risk score for each patient, using the first principal component. Methods used to examine associations between the supervised principal-components risk score, individual genetic lesions and relapse, adverse events, and minimal residual disease are described in the Supplementary Appendix.

RESULTS

COPY-NUMBER ALTERATIONS IN HIGH-RISK ALL

We identified a mean of 8.36 copy-number abnormalities per patient in the original cohort (Table 3 in the Supplementary Appendix), and more than 50 recurring copy-number abnormalities in which the minimal common region of change involved one or few genes (Table 4 in the Supplementary Appendix). The most common deletions involved *CDKN2A/B* (45.7%), the lymphoid transcription-factor genes *PAX5* (31.7%) (Fig. 1 and Table 6 in the Supplementary Appendix) and *IKZF1* (28.6%) (Fig. 2 and Table 8 in the Supplementary Appendix), *ETV6* (also known as TEL) (12.7%), *RB1* (11.3%), and *BTG1* (10.4%).

Twenty-two patients had 27 *PAX5* sequence mutations (Table 7 in the Supplementary Appendix). The most frequent mutation was the previously identified P80R mutation in the paired domain of PAX5 that attenuates the DNA-binding and transactivating activity of PAX5[3] (Fig. 1A). Several novel paired-domain missense (R59G, T75R) and transactivating domain splice-site and frameshift mutations were identified. Each of the paired-domain mutations is predicted to result in impaired binding of PAX5 to DNA, or disruption of the interaction of PAX5 with ETS1, which is required for high-affinity binding of PAX5 to target DNA sequences[21] (Fig. 1B).

Among the 221 patients, the entire *IKZF1* locus was deleted in 16 (Tables 4 and 8 and Fig. 2 in the Supplementary Appendix); in 47 additional patients, a subgroup of exons or the genomic region immediately upstream of *IKZF1* was deleted. In 20 of these 47 patients, there was a deletion of coding exons 3 through 6, which results in expression of a dominant-negative form of IKAROS, Ik6, which lacks all N-terminal, DNA-binding zinc fingers.[5] We also identified six novel missense, frameshift, and nonsense *IKZF1* mutations (Fig. 1C), each of which is predicted to impair IKAROS function. A mutation of G158 is known to attenuate the DNA-binding activity of IKAROS,[22] and thus the G158S mutation we identified would probably act as a dominant-negative IKAROS allele. Overall, 66.5% of the patients with high-risk ALL had at least one mutation of genes regulating B lymphoid development (Tables 4 and 9 in the Supplementary Appendix), with significant variation in the frequency of lesions among ALL subtypes (Table 10 in the Supplementary Appendix).

ASSOCIATIONS WITH OUTCOME

Using supervised principal-components analysis of the original cohort, we identified associations between the copy-number status of 20 genes and treatment outcome (Table 11 in the Supplementary Appendix). The risk score based on the supervised principal-components analysis was significantly associated with poor outcome in the validation cohort. The 10-year incidence of events among patients who were predicted to be at high risk according to the supervised principal-components analysis was 56.9% (95% confidence in-

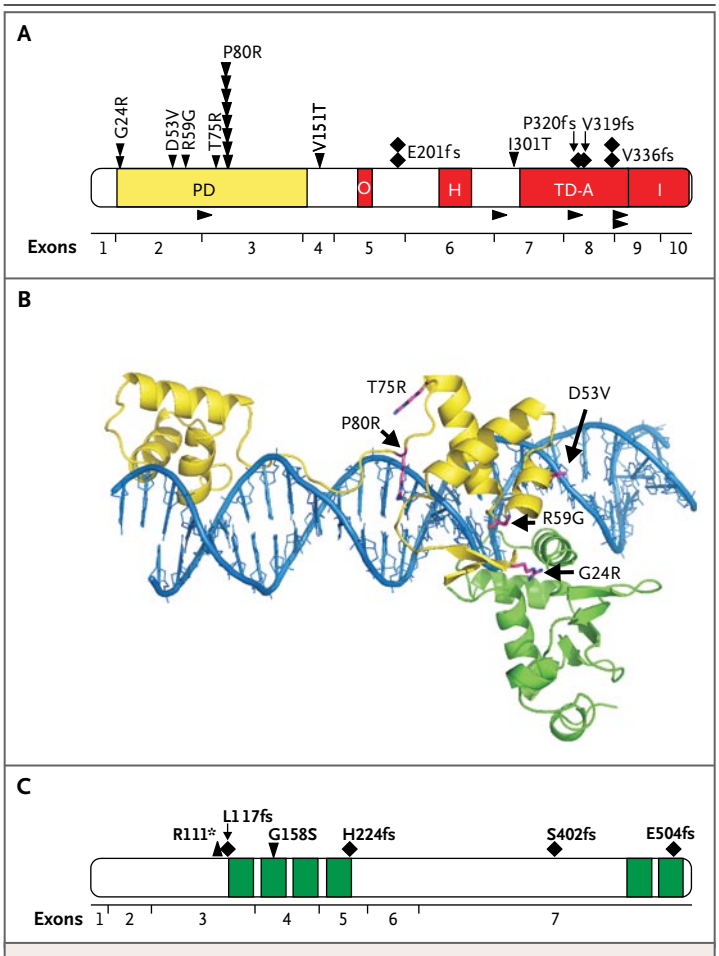

Figure 1. *PAX5* and *IKZF1* Sequence Mutations in High-Risk ALL in the Original Cohort.

Panel A shows the functional domains of PAX5 and the location of missense mutations (arrowheads pointing down), frameshift mutations (diamonds), and splice-site mutations (arrowheads pointing to the right) detected in this study. In the domains, A denotes activating, H homeodomain, I inhibitory, O octapeptide domain, PD paired domain, and TD transactivating domain. Panel B shows structural modeling of the location of PAX5 paired-domain mutations. The DNA double helix is blue, the bipartite PAX5 paired domain is yellow, and ETS1, which interacts with and increases the affinity of DNA binding of PAX5, is green. Each mutation is predicted to disrupt the normal interaction of PAX5 with DNA, ETS1, or both. G24R is predicted to alter the flexibility of the DNA binding loop and interfere with the interaction of PAX5 with ETS1. D53V aligns R56, which in turn directly contacts DNA. R59G occurs at the junction with ETS1 and DNA and is likely to increase flexibility and destabilize both interactions. T75R clashes and causes electrostatic repulsion at R71, which is adjacent to the DNA binding site, and P80R has a direct effect on DNA binding, as previously described.[3] Panel C shows the primary structure of IKAROS and the location of the six zinc fingers (green) and missense (arrowhead pointing down), frameshift (diamonds), and nonsense (arrowhead pointing up) mutations.

terval [CI], 41.5 to 72.3), as compared with 26.8% (95% CI, 19.5 to 34.1) among patients who were predicted to be at low risk (P=0.001) (Fig. 2A). The 10-year incidence of relapse was 47.0% (95% CI, 31.9 to 62.0) among high-risk patients, as compared with 24.6% (95% CI, 17.5 to 31.7) among low-risk patients (P=0.003) (Fig. 2B). Conversely, with the use of the validation cohort as the training set, a supervised principal-components predictor was identified that was associated with poor outcome in the original cohort. The 5-year incidence of adverse events among high-risk patients was 75.0% (95% CI, 59.7 to 90.3), as compared with 27.0% (95% CI, 19.4 to 34.6) among low-risk patients (P<0.001) (Fig. 2C). The 5-year incidence of relapse was 73.8% (95% CI, 58.4 to 89.3) among high-risk patients, as compared with 25.0% (95% CI, 17.6 to 32.4) among low-risk patients (P<0.001) (Fig. 2D).

Of the genetic alterations that were significantly associated with the supervised principal-components predictor in the original cohort (Table 11 in the Supplementary Appendix), only *IKZF1* was also significantly associated with the predictor defined in the validation cohort (Table 12 in the Supplementary Appendix). Deletion or mutation of *IKZF1* was significantly associated with an increased risk of relapse and adverse events in both cohorts (Table 1 and Fig. 3A and 3B, and Tables 13 through 15 in the Supplementary Appendix). *IKZF1* deletions were also associated with a poor outcome in patients with *BCR-ABL1*–negative ALL in the validation cohort (Fig. 3C). Furthermore, alteration of *IKZF1* had an independent association with outcome after adjusting for age, leukocyte count at presentation, and cytogenetic subtype (Table 15 in the Supplementary Appendix). Deletions of *EBF1* and *BTLA/CD200* were associated with a poor outcome only in the original cohort. Although a high cumulative number of genetic alterations targeting B-cell development per patient was also associated with a poor outcome (Tables 13 through 15 in the Supplementary Appendix), no independent association between *PAX5* lesions and outcome was observed in either cohort.

ASSOCIATIONS WITH MINIMAL RESIDUAL DISEASE

Consistent with previous data,[8,15,16] elevated levels of minimal residual disease were strongly associated with an increased risk of relapse in both cohorts (at day 8 and day 29 in the original cohort and at day 19 and day 46 in the validation cohort) (P<0.001 for both comparisons). *IKZF1* and *EBF1* alterations were strongly associated with elevated levels of minimal residual disease at day 29 in the original cohort. Of 67 patients with de-

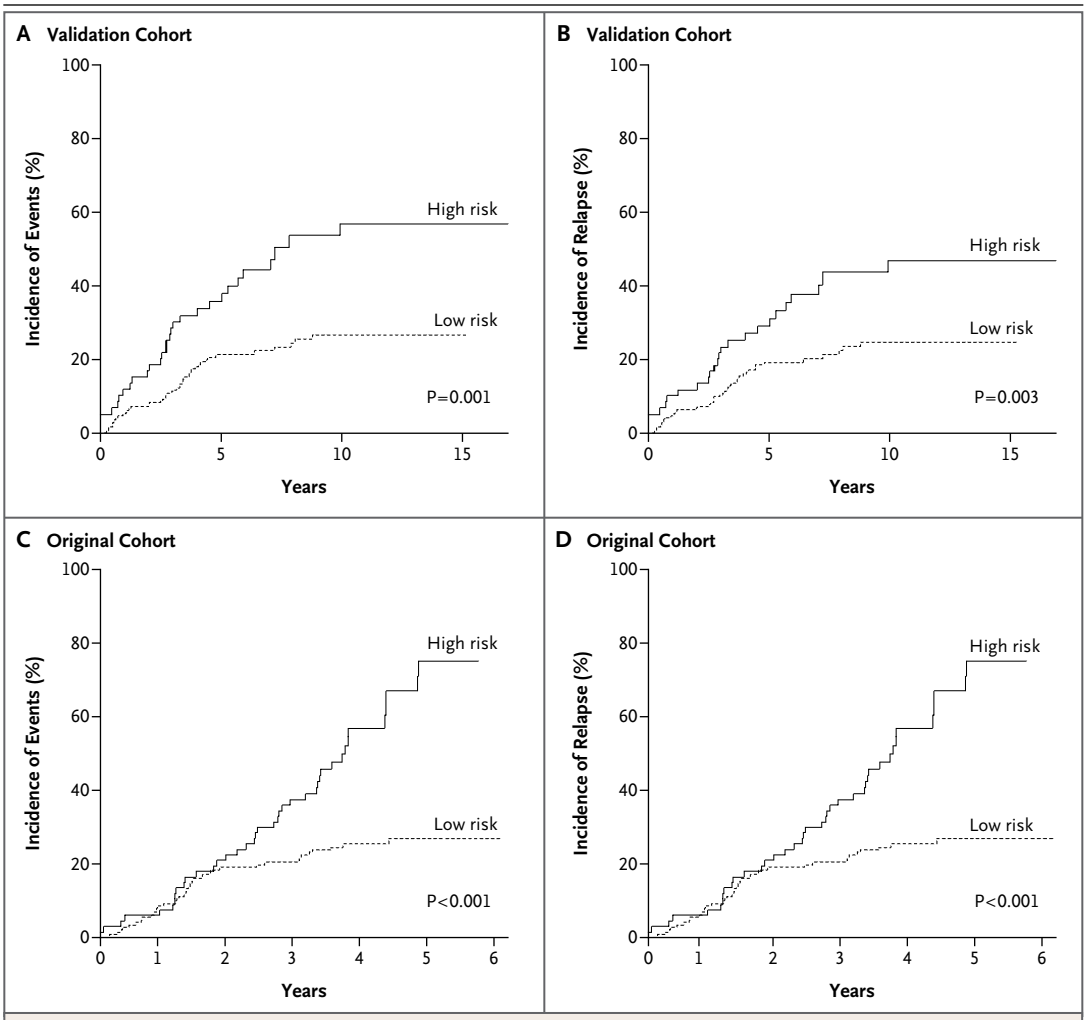

Figure 2. Associations between DNA Copy-Number Abnormality Predictors and Outcome in the Original and Validation Cohorts.

Panel A shows the cumulative incidence of any event (relapse, death, or second malignant condition), and Panel B shows the cumulative incidence of any relapse among patients in the validation cohort after risk stratification with the use of the copy-number abnormality predictor based on data from the original cohort. Panel C shows the cumulative incidence of any event, and Panel D shows the cumulative incidence of relapse in the original cohort after risk stratification with the use of the copy-number abnormality predictor based on data from the validation cohort. High risk refers to patients who are predicted to be at high risk for events or relapse, and low risk refers to patients who are predicted to be at low risk for events or relapse.

leted or mutated *IKZF1*, 16 (23.9%) had high-level (>1%) minimal residual disease at day 29, as compared with 6.6% of patients without this abnormality (P=0.001) (Table 2, and Table 17 in the Supplementary Appendix). These associations remained significant in multivariable analyses adjusted for age, leukocyte count at presentation, and genetic subtype (odds ratio for the association of *EBF1* alterations with elevated levels of minimal residual disease, 9.0; P<0.001; odds ratio for *IKZF1* alterations, 3.71; P<0.001) (Table 18 in the Supplementary Appendix). The associations of *IKZF1* abnormalities with relapse and adverse events remained significant after adjusting for age, leukocyte count, subtype, and minimal residual disease in this cohort (Table 19 in the Supplementary Appendix).

IKZF1 alterations were also associated with outcome in the subgroup of 160 patients for whom we had data on minimal residual disease in the validation cohort (Tables 20 and 21 in the Supplementary Appendix). A deletion or mutation of *IKZF1* was strongly associated with elevated levels of minimal residual disease in this subgroup

Table 1. Alterations in *IKZF1*, *EBF1*, and *BTLA/CD200* and Outcome in the Original and Validation Cohorts.*

Outcome and Alteration	Original Cohort (N=221)				Validation Cohort (N=258)			
	All Patients	Patients with Event	Incidence at 4 or 5 Yr†	P Value	All Patients	Patients with Event	Incidence at 10 Yr	P Value
	no.		%		*no.*		%	
Hematologic relapse								
IKZF1 alteration								
No	154	19	14.0±3.1		210	38	22.5±3.5	
Yes	67	27	55.2±8.6	<0.001	48	19	46.3±8.4	0.002
EBF1 alteration								
No	204	34	22.5±4.1		246	56	28.7±3.6	
Yes	17	12	78.0±12.6	<0.001	12	1	8.3±8.4	0.26
BTLA/CD200 alteration								
No	208	39	18.3±2.9		242	55	28.7±3.6	
Yes	13	7	38.5±14.4	0.003	16	2	13.0±9.0	0.35
Any relapse								
IKZF1 alteration								
No	154	36	25.2±3.8		210	44	25.6±3.6	
Yes	67	39	73.4±8.0	<0.001	48	20	48.4±8.4	0.004
EBF1 alteration								
No	204	62	36.7±4.4		246	62	31.3±3.6	
Yes	17	13	83.9±11.7	<0.001	12	2	16.7±11.3	0.56
BTLA/CD200 alteration								
No	208	64	30.7±3.4		242	62	31.5±3.7	
Yes	13	11	69.2±13.9	<0.001	16	2	13.0±9.0	0.25
Any event								
IKZF1 alteration								
No	154	39	27.1±3.9		210	48	27.6±3.7	
Yes	67	40	74.6±7.9	<0.001	48	25	60.5±8.4	<0.001
EBF1 alteration								
No	204	66	38.7±4.5		246	71	35.5±3.8	
Yes	17	13	83.9±11.7	<0.001	12	2	16.7±11.3	0.40
BTLA/CD200 alteration								
No	208	68	32.6±3.3		242	69	35.2±3.8	
Yes	13	11	69.2±13.9	<0.001	16	4	25.5±11.5	0.83

* Plus–minus values are means ±SE. An event was defined as hematologic relapse, any relapse, a second malignant condition, or death. Associations between genetic alterations of the top-ranked genes (*IKZF1*, *EBF1*, and *BTLA/CD200*) in the supervised principal-components outcome predictor in the original cohort and outcome in the original and validation cohorts are listed. Only the *IKZF1* alteration was associated with outcome in both cohorts. *MKKS/C20orf94* was also associated with the supervised principal-components predictor in the original cohort, but it was not significantly associated with relapse and is not listed.

† Data on the incidence at 5 years are shown for *IKZF1* alteration and *EBF1* deletion, and data on the incidence at 4 years are shown for *BTLA/CD200* deletion.

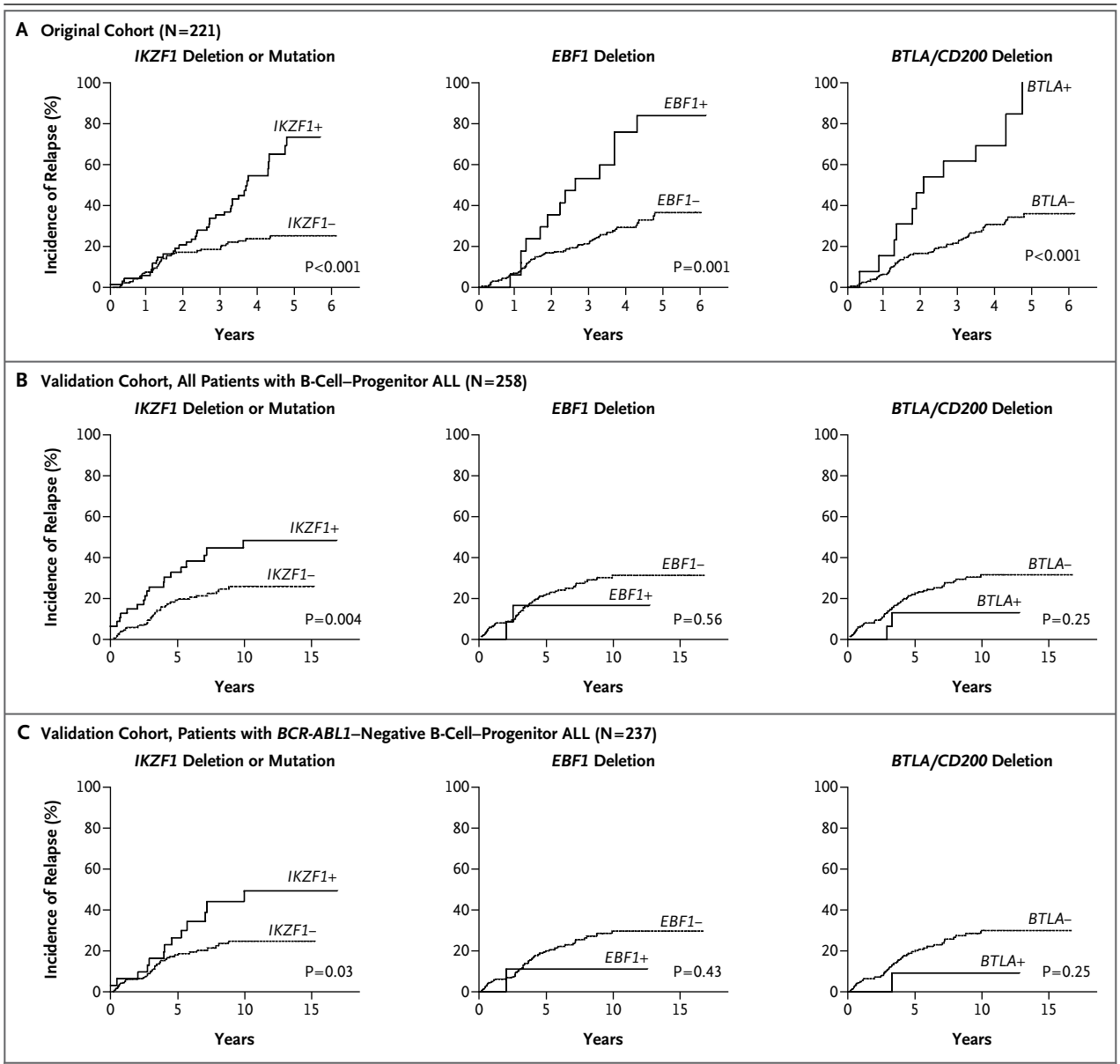

Figure 3. Genetic Alterations of *IKZF1*, *EBF1*, and *BTLA/CD200* and the Cumulative Incidence of Any Relapse in the Original Cohort.

Panel A shows the cumulative incidence of relapse in the entire original cohort of patients with B-cell–progenitor ALL according to *IKZF1*, *EBF1*, and *BTLA/CD200* alteration status. Panel B shows the cumulative incidence of relapse in the entire validation cohort, including patients with *BCR-ABL1*–positive B-cell–progenitor ALL. Panel C shows the cumulative incidence of relapse in the validation cohort after exclusion of patients with *BCR-ABL1*–positive B-cell–progenitor ALL. Only *IKZF1* alterations were associated with poor outcome in both the original and validation cohorts. Five-year estimates of relapse are shown in the original cohort, and 10-year estimates are shown in the validation cohort. P values are calculated with the use of Gray's test.

of patients. High levels of residual disease (≥1.0%) at day 19 were detected in 13 patients with a *IKZF1* deletion or mutation (61.9%), as compared with 9.3% of patients without deletion or mutation of *IKZF1* (P<0.001) (Table 2, and Table 22 in the Supplementary Appendix). This association was also observed for minimal residual disease at day 46 (33.3% vs. 0.7%, P<0.001) (Table 2, and Table 23 in the Supplementary Appendix). *IKZF1* status was also associated with minimal residu-

Table 2. Associations between *IKZF1* Alterations and Levels of Minimal Residual Disease.

Cohort	*IKZF1* Deletion or Mutation	Patients	Level of Minimal Residual Disease*			P Value
			Low	Intermediate	High	
		no.	*no. of patients (%)*			
Original						
Day 8	No	133	26 (19.5)	50 (37.6)	57 (42.9)	
	Yes	63	7 (11.1)	17 (27.0)	39 (61.9)	0.04
Day 29	No	137	100 (73.0)	28 (20.4)	9 (6.6)	
	Yes	67	31 (46.3)	20 (29.9)	16 (23.9)	0.001
Validation						
Day 19	No	140	69 (49.3)	58 (41.4)	13 (9.3)	
	Yes	21	2 (9.5)	6 (28.6)	13 (61.9)	<0.001
Day 46	No	139	119 (85.6)	19 (13.7)	1 (0.7)	
	Yes	21	7 (33.3)	7 (33.3)	7 (33.3)	<0.001

* In the original cohort, a low level of minimal residual disease was 0.01% or less and a high level was greater than 1.0%. In the validation cohort, a low level of minimal residual disease was less than 0.01% and a high level was 1.0% or greater.

al disease at both day 19 (P=0.001) and day 46 (P=0.001) in patients in the *BCR-ABL1*–negative validation cohort (Tables 24 and 25 in the Supplementary Appendix).

GENE-EXPRESSION PROFILING OF HIGH-RISK ALL

The association between *IKZF1* alterations and outcome in both cohorts, as well as previous data showing that deletion of *IKZF1* is frequent in *BCR-ABL1* ALL,[5] suggests that IKAROS abnormalities are important in the pathogenesis of both *BCR-ABL1*–positive B-cell–progenitor ALL and *BCR-ABL1*–negative ALL that is associated with a poor outcome. To explore this possibility, we used gene-set enrichment analysis to compare the gene-expression signatures of patients with ALL who had a poor outcome in the original and validation cohorts. We also used this form of analysis to compare the gene-expression signatures of *BCR-ABL1*–positive ALL and *BCR-ABL1*–negative ALL associated with a poor outcome in the original cohort. This analysis revealed a significant similarity of signatures in the patients with ALL who had a poor outcome in the original and validation cohorts (Fig. 3A and 3B in the Supplementary Appendix).

Using gene-set enrichment analysis, we also observed a highly significant similarity between the signature of high-risk, *BCR-ABL*–negative ALL (derived from the original cohort) and the signature of *BCR-ABL1*–positive ALL in the validation cohort (Fig. 3C and 3D in the Supplementary Appendix). Moreover, 61 of the 100 most differentially expressed genes in patients with ALL with a poor outcome in the original cohort were present in patients with the *BCR-ABL1* signature in the validation cohort (at a false discovery rate of 5%), indicating substantial similarity between the two signatures. These findings suggest that genetic alterations of *IKZF1* influence the transcriptome of both *BCR-ABL1*–positive ALL and *BCR-ABL1*–negative ALL with a poor outcome. We also observed the enrichment of genes up-regulated in hematopoietic stem cells and progenitor cells[23] in patients with ALL who had a poor outcome in both the original and validation cohorts (Table 26 and Fig. 3E in the Supplementary Appendix) and a relative lack of expression of genes mediating B-lymphocyte–receptor signaling and development[24] in patients with a poor outcome in the original cohort (Table 27 and Fig. 3F in the Supplementary Appendix), suggesting that *IKZF1* alterations result in developmental arrest and impaired B-cell development. Finally, gene-set analysis[18] with the use of time to first event as the phenotype showed that the *BCR-ABL1* signature was the gene set most strongly predictive of a poor outcome in the original (*BCR-ABL1*–negative) cohort (P<0.001).

DISCUSSION

Accurate risk stratification is critical for ensuring that patients with high-risk ALL receive treat-

ment of appropriate intensity and that low-risk patients are spared unnecessary toxic effects. Current risk stratification is based primarily on clinical variables, immunophenotype, detection of sentinel cytogenetic or molecular lesions, and early response to therapy.[1] However, a substantial proportion of patients who have a relapse have no known poor-risk factors at the time of diagnosis.

We used high-resolution, genomewide copy-number analysis to identify genetic lesions associated with clinical outcome. Most striking was the strong association between deletions or mutations of *IKZF1* and a poor outcome in two independent cohorts notable for different sample composition and treatment schedules. In multivariate analysis, the association between *IKZF1* status and outcome was independent of age, leukocyte count at presentation, cytogenetic subtype, and levels of minimal residual disease; this indicates that detection of *IKZF1* alterations at diagnosis might be useful in identifying patients with a high risk of treatment failure. Moreover, the gene-expression signatures of patients with poor-outcome (*IKZF1*-deleted) ALL in the original and validation cohorts were very similar to each other and to the signature of *BCR-ABL1*–positive ALL, a subtype of ALL in which *IKZF1* deletion is very common. Since *BCR-ABL1* ALL has a poor prognosis, these findings suggest that the mutation of *IKZF1* is a key determinant of a poor outcome both in patients with *BCR-ABL1*–positive and patients with *BCR-ABL1*–negative disease. The similarity of the gene-expression signatures of *BCR-ABL1*–negative ALL with a mutation of *IKZF1* and *BCR-ABL1*–positive ALL raises the possibility that patients with *BCR-ABL1*–negative ALL, deletion of *IKZF1*, and a poor outcome may have hitherto unidentified activating mutations in tyrosine kinases.

IKAROS is a transcription factor with well-established roles in lymphopoiesis and cancer.[25] Normal IKAROS contains four N-terminal zinc fingers, which are required for DNA binding, and two C-terminal zinc fingers that mediate dimerization of IKAROS with itself and with other IKAROS family members. The development of all lymphoid lineages requires IKAROS,[26] and in mice that are heterozygous for a dominant-negative *Ikzf1* mutation, aggressive T-lineage hematopoietic disease develops.[27] *Ikzf1* is also a common target of integration in retroviral mutagenesis studies in mice.[28]

Alternative *IKAROS* transcripts have been detected in normal hematopoietic cells and leukemic blasts.[25] Isoforms lacking most or all of the N-terminal zinc fingers have attenuated DNA-binding capacity but retain their ability to undergo homodimerization and heterodimerization, and they thus act as dominant-negative inhibitors of IKAROS.[29] Previous studies have shown expression of these aberrant IKAROS isoforms in ALL.[25] Recently, we reported a very frequent deletion of *IKZF1* in *BCR-ABL1*–positive ALL and lymphoid blast crisis of chronic myeloid leukemia, suggesting that perturbation of IKAROS is a key event in the pathogenesis and progression of *BCR-ABL1* leukemia.[5] Moreover, there was complete correlation between the extent of genomic deletion and the expression of aberrant IKAROS isoforms.[5] For example, all patients expressing the dominant-negative Ik6 isoform, which lacks coding exons 3 through 6 and all N-terminal zinc fingers, had genomic deletions of exons 3 through 6.[5]

The present study shows that *IKZF1* alterations occur in a substantial proportion of patients with *BCR-ABL1*–negative B-cell–progenitor ALL, predominantly in patients without other common recurrent cytogenetic abnormalities (38.8% of patients in the original cohort and 22.8% of the patients in the validation cohort with normal or miscellaneous karyotypic abnormalities had alterations of *IKZF1*). As in *BCR-ABL1*–positive ALL, *IKZF1* deletions involved either the entire locus or sets of exons, and they are predicted to result in either haploinsufficiency or the expression of dominant-negative IKAROS isoforms. Moreover, we have identified sequence mutations of *IKZF1* in ALL that are predicted to result in the loss of normal IKAROS function or expression of a novel dominant-negative isoform, G158S.

Using gene-set enrichment analysis, we found enrichment of hematopoietic stem-cell and progenitor genes and underexpression of B lymphoid genes in patients with ALL who had a poor outcome. This finding is consistent with the requirement for IKAROS in lymphoid development[26] and the demonstration that expression of dominant-negative IKAROS isoforms impairs B lymphoid differentiation.[30] Together, these data suggest that attenuation of normal IKAROS activity and the resulting block in lymphoid maturation render leukemic cells relatively resistant to eradication by chemotherapy. The clinical consequences of enrichment for genes that are characteristic of

leukemia-initiating cells or stem cells, including their inherent drug-resistant mechanisms, remain to be determined.[31]

We did not find an association between clinical outcome and extensively studied loci such as *CDKN2A/B*[32,33] or *PAX5* status, despite the finding that *PAX5* alterations were the most common lesions in the B-cell–differentiation pathway in both cohorts. *PAX5* alterations may be important in establishing the leukemic clone, whereas alterations of *IKZF1* may also contribute to resistance to chemotherapy. This finding is supported by recent data showing that *IKZF1* alterations also emerge as new genetic alterations at the time of relapse in ALL.[34] In summary, we identified an association between alterations of *IKZF1* and the clinical outcome in B-cell–progenitor ALL in childhood. Integrated genomic analysis suggests that *IKZF1* contributes directly to treatment resistance in ALL. These results provide a rationale for the integration of *IKZF1* status in the evaluation of patients with ALL.

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