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ORIGINAL REPORT

IKZF1 (Ikaros) Deletions in *BCR-ABL1*–Positive Acute Lymphoblastic Leukemia Are Associated With Short Disease-Free Survival and High Rate of Cumulative Incidence of Relapse: A GIMEMA AL WP Report

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
The causes of the aggressive nature of *BCR-ABL1*–positive adult acute lymphoblastic leukemia (ALL) are unknown. To identify, at the submicroscopic level, oncogenic lesions that cooperate with *BCR-ABL1* to induce ALL, we performed an investigation of genomic copy number alterations using single nucleotide polymorphism array, genomic polymerase chain reaction, and sequencing of candidate genes.

Patients and Methods
Eighty-three patients with de novo adult Philadelphia chromosome (Ph) –positive ALL were enrolled onto institutional (n = 17) or Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto Working Party delle Leucemia Acute (n = 66) clinical trials. Treatments included tyrosine kinase inhibitor (TKI) alone, conventional chemotherapy, or a combination of TKI and chemotherapy.

Results
A 7p12 deletion of *IKZF1* (Ikaros) was identified in 52 (63%) of 83 patients. The pattern of deletion varied among different patients, but the two most common deletion types were loss of exons 4 to 7 in 31 (37%) of 83 patients and loss of exons 2 to 7 in 17 (20%) of 83 patients. Disease-free survival (DFS) was shorter in patients with *IKZF1* deletion versus patients with *IKZF1* wild type (10 *v* 32 months, respectively; *P* = .02). Furthermore, a significantly shorter cumulative incidence of relapse was recorded in patients with *IKZF1* deletion versus patients with *IKZF1* wild type (10.1 *v* 56.1 months, respectively; *P* = .001). Multivariate analysis confirmed the negative prognostic impact of *IKZF1* deletion on DFS (*P* = .04).

Conclusion
We conclude that *IKZF1* deletions are likely to be a genomic alteration that significantly affects the prognosis of Ph-positive ALL in adults.

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INTRODUCTION

The Philadelphia chromosome (Ph),[1] arising from a reciprocal translocation between chromosomes 9 and 22,[2] was the first defined cytogenetic abnormality recognized as linked to both chronic myeloid leukemia (CML) and Ph-positive acute lymphoblastic leukemia (ALL). This translocation fuses the *ABL1* oncogene on chromosome 9 to a breakpoint cluster region (*BCR*) from chromosome 22. It generates the constitutively activated Bcr-Abl tyrosine kinase, which is responsible for both acute and chronic disease.[3-5] In CML, a p210 *BCR-ABL* isoform is initially expressed in hematopoietic stem cells capable of giving rise to both differentiated myeloid and lymphoid progeny, whereas in de novo Ph-positive ALL, the expression of either of two alternative p185 and p210 isoforms is restricted to the B-cell lineage.[6] CML typically presents as an indolent myeloproliferative disease (so-called chronic phase) that, if untreated, invariably evolves to blast crisis in which poorly differentiated malignant myeloid or lymphoid blast cells become resistant to any therapy approach.

The outcome of patients with Ph-positive ALL has improved dramatically with current therapies that include the use of tyrosine kinase inhibitors (TKIs) such as imatinib,[7] nilotinib,[8,9] or dasatinib.[10]

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Complete hematologic remissions (CHR) can be obtained in 98% to 100% of patients treated with TKI alone[7] or in association with conventional chemotherapy,[11] but relapse is an expected event in the majority of patients.[9] Current approaches to risk classification are based on well-established clinical parameters, aberrant expression of antigens on the surface of blast cells by immunophenotypic analysis, early detection of minimal residual disease persistence after therapy, and genetic lesions and aberrant expression profiles of the leukemic population.

In the past, before the advent of TKIs, the outcome after chemotherapy for patients with Ph-positive ALL was gloomy. Complete remission (CR) rates with conventional and intensive ALL regimens ranged from 60% to 90%.[12] The long-term disease-free survival (DFS) rate was approximately 10% in the absence of allogeneic stem-cell transplantation (SCT) procedures. Median survival time ranged from 8 to 16 months as a result of relapse-related mortality. Improved CR rates with the more intensive regimens did not translate into an increase in durability of response. Our group has shown that after high-dose anthracycline chemotherapy, chemotherapy-sensitive patients who achieved at least a 3-log reduction in *BCR-ABL1* transcripts by quantitative real-time polymerase chain reaction (RT-PCR) after consolidation chemotherapy had 2-year DFS and overall survival (OS) rates of 27% and 48%, respectively, which are not dissimilar from the rates observed after allogeneic SCT in first CR.[13] None of the patients who had less than a 3-log reduction in *BCR-ABL* transcripts were alive at 2 years, suggesting that subcategories of Ph-positive ALL exist.

Using high-resolution single nucleotide polymorphism (SNP) arrays, Mullighan et al[14] found an exceptionally high loss of the *IKZF1* gene on 7p12, which encodes the transcription factor Ikaros in 84% of *BCR-ABL1* ALL, but not in chronic-phase CML, and in 28% of *BCR-ABL1*–negative B-cell ALL.[15] Ikaros is a member of a family of zinc finger–containing transcription factors. Like many such genes, it possesses DNA domains involved in homo- and heterodimer formation, with internal DNA domains coding for zinc fingers. Ikaros undergoes several splice variations, and it is thought that the mix of these splice variants influences Ikaros function.[16-19] In pediatric B-cell progenitor ALL, including patients with common aneuploidies and patients with recurring translocations, *IKZF1* deletions correlated significantly with an increased frequency of relapse at 5 and 10 years and with resistance to chemotherapy.[15] However, the prognostic value of *IKZF1* deletions in adults patients with *BCR-ABL1*–positive ALL is still lacking.

In our study, using high-resolution interrogation of genomic copy number alterations (NspI 250K and SNP6.0; Affymetrix, Santa Clara, CA), deletions involving only the *IKZF1* gene were identified in 52 (63%) of 83 adult patients. It noteworthy that in eight (10%) of 83 patients, we observed a loss of all *IKZF1* as a result of monosomy of chromosome 7 reaching a global loss of *IKZF1* in 72% of patients with *BCR-ABL1*–positive ALL. We correlated focal *IKZF1* deletions with the clinical outcome, OS, cumulative incidence of relapse (CIR), relapse rate after obtaining a CHR, and DFS and demonstrated that Ikaros deletions represent the most important genetic prognostic factor thus far described in Ph-positive ALL.

PATIENTS AND METHODS

Patients

Between April 1996 and April 2008, 83 patients (46 men and 37 women; median age, 56 years; range, 18 to 78 years) with de novo Ph-positive ALL provided informed consent for molecular and clinical analysis and were studied for *IKZF1* deletions (Table 1). Sixty-six patients (80%) were enrolled onto Gruppo Italiano Malattie Ematologiche dell'Adulto clinical trials (LAL0201-B protocol, n = 18; LAL2000 protocol, n = 15; LAL1205 protocol, n = 33), whereas 17 patients (20%) were enrolled onto institutional protocols (Appendix Table A1, online only). Details of therapy schemes are provided in the Appendix (online only).

Only five patients underwent an allogeneic bone marrow transplantation in CHR as consolidation therapy; they were not censored for statistical analysis (HLA identical, n = 1; matched unrelated donor, n = 3; allogeneic, n = 1). Fifty-seven patients received TKI treatment; 13 patients (LAL1205) received TKI only during induction, 38 patients received TKI during induction and during maintenance (LAL1205, n = 20; LAL0201-B, n = 18), and six patients received TKI only during maintenance (LAL2000). At diagnosis, all patients were found to be *BCR-ABL1* positive. The percentages of patients with *BCR-ABL* fusion transcripts corresponding to p210 versus p190 versus p190+p210 were 26%, 63%, and 13%, respectively. All 83 patients with *BCR-ABL1*–positive ALL were analyzed by SNP array and by genomic PCR analysis.

SNP Microarray Analysis

Genomic DNA was extracted using the DNA Blood Mini Kit (Qiagen, Valencia, CA) from mononuclear cells isolated from peripheral-blood or bone marrow aspirate samples by Ficoll gradient centrifugation. Samples were genotyped with Affymetrix NspI 250K and SNP6.0 arrays (Affymetrix) according to the manufacturer's instructions. CEL files and corresponding SNP genotype call files were generated using Affymetrix GeneChip Genotyping Analysis Software (GTYPE) version 4.0. Affymetrix CEL files were then analyzed for genomic copy number variations using the Partek Genomic Suite software (Partek, St Louis, MO).

Table 1. Patient Demographics and Clinical Characteristics of Patients With Ph-Positive Acute Lymphoblastic Leukemia

Patient Demographics and Clinical Characteristics	No. of Patients (N = 83)	%
Age, years		
Median	56.1	
Range	13.2-78.0	
Blasts, %		
Median	90	
Range	28-99	
Sex		
Male	46	55.42
Female	37	44.58
Study		
LAL2000	15	18.07
LAL1205	33	44.76
LAL0201-B	18	21.69
Institutional protocol	17	20.48
Leukocytes, /μL		
Median	30.0	
Range	1.4-302.0	
Molecular		
BCR-ABL-positive P210	21	26
BCR-ABL-positive P190	52	63
BCR-ABL-positive P210 and P190	10	12

Abbreviation: PH, Philadelphia chromosome.

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IKZF1 *RT-PCR and Gene Expression Analysis*

Total cellular RNA was extracted from mononuclear cells using the RNeasy total RNA isolation kit (Qiagen), according to the instructions of the manufacturer, and 1 μg of the total RNA sample was used for cDNA synthesis with Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA), as previously described.[20]

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization analysis was performed as previously described.[21] The whole chromosome paints used for chromosome 7, derived from flow-sorted chromosomes, were a gift of the Sanger Institute (Cambridge, United Kingdom; Dr Nigel Carter). Fosmid probes specific for the *IKZF1* gene (G248P800745C8 [chr7:50,381,496-50,422,338] and G248P87926C7 [chr7:50,418,455-50,458,507]), as well as a bacterial artificial chromosome probe specific for *BCR* (RP11-164N13 [chr22:21,897,904-22,091,572]), were properly selected accordingly to the latest release (March 2006) of the University of California, Santa Cruz (UCSC) Human Genome Browser (http://genome.ucsc.edu/).

Statistical Analysis

The primary study end points were achievement of CR, duration of first CR (in terms of DFS and CIR), and OS. Median follow-up time was estimated by reversing the codes for the censoring indicator in a Kaplan-Meier analysis.[22] Details on statistical analysis are provided in the Appendix.

RESULTS

SNP Microarray Analysis Detects Frequent and Recurrent Deletions in IKZF1 *Gene, and Two Types of Deletions Account for Most* BCR-ABL1*–Positive ALL*

Using high-resolution SNP array, we profiled the genomes of 83 patients with *BCR-ABL1*–positive ALL and found that 7p12 deletions involving the *IKZF1* gene are the most frequent somatic copy number alterations (52 of 83 patients, 63%) in *BCR-ABL1*–positive ALL. *IKZF1* encodes the transcription factor Ikaros that is required for the earliest stages of lymphoid lineage commitment and acts as tumor suppressor in mice.[16] *IKZF1* deletions have been confirmed by fluorescence in situ hybridization analysis[14](Fig 1). These data are in agreement with the previously reported frequencies, mostly in pediatric patients.[14] As we previously reported,[23] we characterized and mapped all genomic breakpoints to recognize that two major deletions occur in the *IKZF1* gene. The first one was identified in 31 (37%) of 83 patients and was characterized by loss of exons 4 to 7 (Δ4-7) with breakpoints occurring in introns 3 and 7 on chromosome 7p12. As reported, the extent of the deletion correlated with the expression of the dominant-negative isoform Ik6 with cytoplasmatic localization and oncogenic activity.[20] The second deletion was identified in 17 (20%) of 83 patients and involved exons 2 to 7 (Δ2-7) with a variable pattern of breakpoints in intron 1 and intron 7 in the same region as those of the Δ4-7 deletion. There was a correlation between the extension of this deletion and the expression of an aberrant untranslated transcript containing only exons 1 and 8. In the remaining patients, the promoter region or exon 8 was also involved in the deletion (Table 2). A variable number of patient-specific nucleotides was inserted at the conjunction.

Recurrent Deletions in IKZF1 *Gene Are Associated With a Worse Prognosis for Patients With* BCR-ABL1*–Positive ALL*

Patient characteristics. All patients were white, and most were Italian. Twenty-one patients (25%) were 60 years of age or older. The median follow-up time was 14.8 months (range, 0.4 to 148.1 months). Initial WBC counts ranged between 14,000 and 302,000/μL (median, 30,000/μL). No patient had symptomatic CNS disease at diagnosis. All patients were evaluable for central review. Central or local institutional

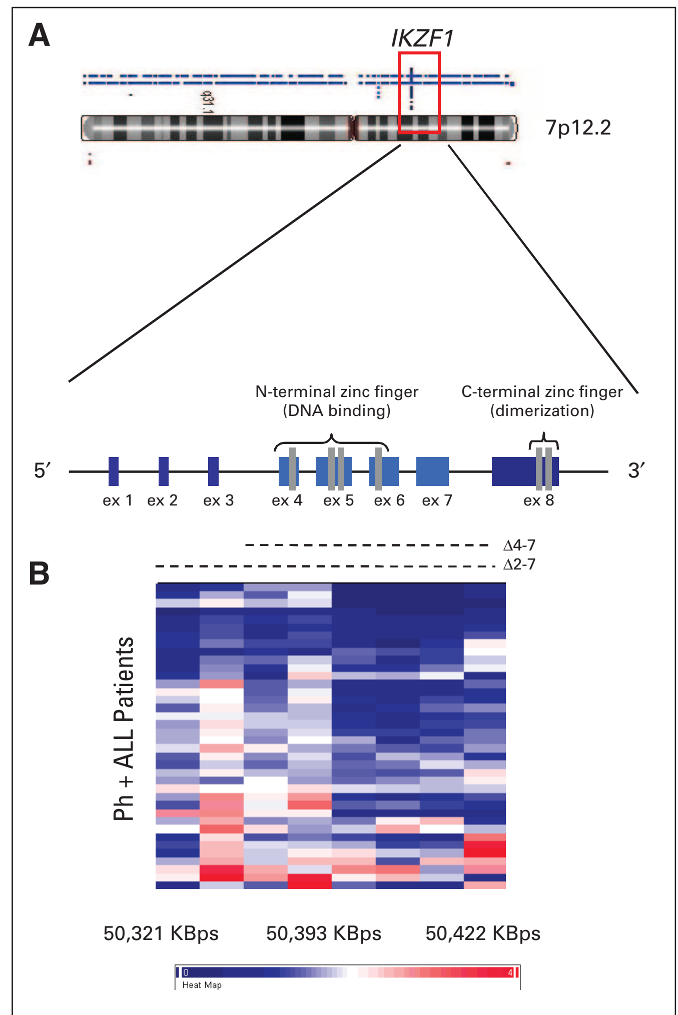

Fig 1. (A) Schematic diagram of the full-length *IKZF1* cDNA with N-terminal zinc fingers that show DNA binding activity and C-terminal zinc fingers that mediate dimerization of the protein. (B) HeatMap representation from Partek Genomic Suite (Partek, St Louis, MO) software of *IKZF1* deletions in patients with Philadelphia chromosome (Ph) –positive acute lymphoblastic leukemia (ALL). Deletions are shown in blue, and gains are shown in red. Each line represents the deletion observed in each case. ex, exon.

Table 2. Results of Molecular Assays for Type of *BCR-AB1* Rearrangement and *IKZF1* Status in Patients With Ph-Positive ALL

IKZF1 Status	No. of Patients (N = 83)	%
IKZF1 wild type	31	37
IKZF1 focal deleted	52	63
IKZF1 Δ4-7 deletion	31	59
IKZF1 Δ2-7 deletion	17	33
Other deletions	4	8

Abbreviations: PH, Philadelphia chromosome; ALL, acute lymphoblastic leukemia.

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immunophenotyping was successfully performed in all patients and confirmed the B-lineage affiliation.

We investigated whether *IKZF1* deletions were equally distributed between age, during time (ie, DNA sample stability/integrity), and between protocols (ie, in single clinical trials). We found an equal distribution of *IKZF1* deletion between all these variables (Appendix Tables A2 and A3, online only).

Remission induction. All patients started induction therapy and consequently were evaluable for CHR (by intention to treat). If the final evaluation of the treatment for a patient was lost, the patient was evaluated at the last visit available (last observation carried forward). One patient never started therapy, and two patients (both on protocol LAL1205) were lost to follow-up. Furthermore, all patients (100%) enrolled onto protocol LAL1205 (dasatinib as front-line therapy) and all patients (100%) enrolled onto LAL0201-B (imatinib and corticosteroids as front-line therapy for elderly patients) obtained a CHR. Because, for this reason, it was not possible to correlate CHR with other parameters, only 31 patients (37.3%) were evaluable for a correlation between CHR and *IKFZ1* deletion (Appendix Table A4, online only). At the end of induction chemotherapy, 25 (81%) of 31 patients attained a CHR, and six patients (19%) were resistant to induction chemotherapy (ie, > 25% blasts persisting in the marrow; data not shown); seven (28%) of 25 patients had *IKZF1* wild type, whereas 18 (72%) of 25 patients had *IKZF1* deletion (P = 1.000), suggesting that *IKZF1* deletion is not associated with a reduced probability of obtaining a CHR. No correlation was found between the type of *IKZF1* deletion (Δ2-7 *v* Δ4-7) and the rate of CHR. A univariate analysis of patient characteristics and their association with outcome was also performed (Appendix Table A5, online only) and demonstrated that they did not influence the CHR.

High Rate of CIR and Short DFS Are Associated With IKZF1 Deletion

The median time of cumulative incidence of relapse (CIR) of the entire population was 12.5 months (Table 3 and Fig 2); for patients with *IKZF1* deletion, the CIR time was 10.1 months, whereas for patients without *IKZF1* deletion, the CIR time was 56.1 months (P = .01). Furthermore, a significantly higher rate of CIR for patients with *IKZF1* deletion (69.1%) versus patients without *IKZF1* deletion (40.4%; P = .0103) was also recognized. Five patients (6%) died in CR without evidence of leukemia relapse; this explains the difference between the CIR and DFS curves.

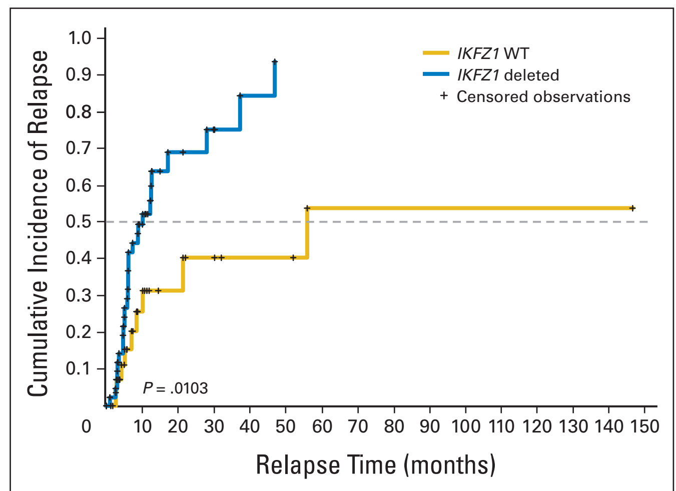

Fig 2. Cumulative incidence of relapse of de novo–treated patients with Philadelphia chromosome–positive acute lymphoblastic leukemia treated with conventional or investigational therapy including tyrosine kinase inhibitor (imatinib or dasatinib) regimens and with *IKZF1* deletion compared with patients treated with the same protocols without *IKZF1* deletion (*IKZF1* wild type [WT]).

The median OS time of the entire population (N = 83) was 49.3 months, with OS rates of 87.9% (95% CI, 81.3% to 95.2%), 71.7% (95% CI, 62.5% to 82.1%), and 54.1% (95% CI, 44.3% to 66.2%) at 12, 24, and 36 months, respectively (P = .7830). This is a long OS for adult patients with Ph-positive ALL, particularly considering the high number of patients older than age 55 years. This prolonged OS is likely related to the fact that more than 50% of the ALL patients were treated with imatinib either as first-line therapy or as maintenance therapy

Table 3. Treatment Outcome and Results of Therapy Related to the *IKZF1* Deletion (univariate analysis)

Outcome	All Patients (N = 83)	Ikaros Status: Patients With *IKZF1* Wild Type (n = 31)	Ikaros Status: Patients With *IKZF1* Deletion (n = 52)	P
CIR				.0103
No. of patients	74	30	44	
CIR at 24 months, %	59.5	40.4	69.1	
95% CI	58.4 to 60.7	37.3 to 43.7	67.5 to 70.7	
Median time, months	12.5	56.1	10.1	
DFS				.0229
No. of patients	74	30	44	
DFS at 24 months, %	38.7	53.9	30.9	
95% CI	33.5 to 44.7	42.2 to 68.8	26.1 to 36.6	
Median time, months	12.5	32.1	10.1	
OS				.7830
No. of patients	83	31	52	
OS at 24 months, %	71.7	66	76.1	
95% CI	62.5 to 82.1	52 to 83.7	65.2 to 88.8	
Median time, months	49.3	33.5	49.3	

Abbreviations: CIR, cumulative incidence of relapse; DFS, disease-free survival; OS, overall survival.

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after obtaining CHR; furthermore, when they experienced relapse, most of the patients were treated with a second-generation TKI, such as dasatinib, nilotinib, or bosutinib, or aurora kinase inhibitors.[24] No statistical differences in OS were found in patients with *IKF1* deletion or wild type (Table 3 and Appendix Fig A1, online only). A univariate analysis of patient characteristics and their association with outcome was also performed (Appendix Table A6, online only), and only age was demonstrated to influence OS. After obtaining a CHR, 37 patients (50.0%) experienced a leukemia relapse. Median DFS time was 12.5 months, with DFS rates of 53.3% (95% CI, 47% to 60.5%), 38.7% (95% CI, 33.5% to 44.7%), and 25.8% (95% CI, 22.1% to 30.1%) at 12, 24, and 36 months, respectively.

A univariate analysis of patient characteristics and their association with outcome was also performed (Appendix Table A7, online only). A shorter time of DFS was found in patients with *IKZF1* deletion compared with wild-type patients (10.1 *v* 32.1 months, respectively; $P = .01$; Fig 3). DFS was not significantly shorter in patients with Δ2-7 versus Δ4-7 (8.8 *v* 12.3 months, respectively). Multivariate analysis confirmed the negative prognostic impact of Ikaros deletion on DFS ($P = .0425$; Appendix Tables A8 and A9, online only). Furthermore, multivariate analysis revealed that the type of *BCR-ABL* (p190+p210 *v* p210 only) is the only factor that influences OS.

DISCUSSION

The Ph chromosome encodes the oncogenic *BCR-ABL1* kinase and defines a subgroup of patients with ALL who have a particularly unfavorable prognosis.[25] The reasons for the aggressive nature of *BCR-ABL1*–positive ALL are still under investigation and have not yet been understood. After an initial relatively good response to conventional chemotherapy, resistance to therapy and chemotherapy-refractory relapse occur. This outcome emphasizes specific genetic differences between Ph-positive ALL and other subgroups of ALL or CML, the other Ph-positive leukemia.

Using high-resolution genomic study of copy number alterations in the largest cohort of adult patients (N = 83) with *BCR-ABL1*–positive ALL reported, to our knowledge, thus far, we found that homozygosis or heterozygosis deletions in the *IKZF1* gene frequently occur in patients with *BCR-ABL1*–positive ALL (63%), as previously documented by Mullighan et al[14] in 21 pediatric and 22 adult *BCR-ABL1*–positive ALL patients. Recently, Mullighan et al[15] reported the prognostic implications of *IKZF1* deletions in children with B-cell progenitor ALL; they determined that *IKZF1* deletions correlated significantly with an increased frequency of relapse at 5 and 10 years and with resistance to chemotherapy. Notably, the *IKZF1* association was independent of *BCR-ABL1* translocations and other risk factors. However, the prognostic implications of *IKZF1* in adults with B-cell progenitor ALL are still lacking. It is possible that similar to pediatric data, *IKZF1* deletions identify a group of adult patients with high-risk ALL with increased risk for relapse. To address this issue, we analyzed genomic copy number abnormalities using SNP microarrays in patients with *BCR-ABL1*–positive ALL and focused our attention on the gene that encodes the transcription factor Ikaros (*IKZF1*) because it was the most frequent genomic copy number abnormality (63%).

In this cohort of patients, no associations were observed between *IKZF1* deletions and achievement of remission in 31 patients who received induction with chemotherapy alone. Deletions of *IKZF1* were found to confer a shorter cumulative median DFS and a higher median CIR in *BCR-ABL1*–positive ALL. Multivariate analysis confirmed the negative prognostic impact of *IKZF1* deletion on DFS ($P = .0425$). It is noteworthy that in a multivariable model with outcome as a dependent variable and study, Ikaros status, WBC, and so on as the independent variables, *IKZF1* deletions still remain the only feature that influences clinical outcome across the different studies. By this analysis, we also observed a significant difference in DFS between LAL0201B (older Ph-positive patients treated with corticosteroid plus imatinib as first-line therapy) and LAL2000 (young patients treated up front with conventional chemotherapy, high-dose cytarabine and mitoxantrone as consolidation, and imatinib as maintenance). It is probable that this difference in DFS could be influenced by age. However, this does not affect the negative prognostic value of *IKZF1* deletion. An interesting finding is the increased risk of relapse and shorter DFS for patients with *IKZF1* deletion. We suppose that this could be justified by two hypotheses; first, the follow-up is still too short, and patients who experience relapse are still alive and do not influence the OS; second, patients who experienced relapse may achieve a second remission after second-generation TKI treatment. However, this second hypothesis will be clarified with longer follow-up.

Why do *IKZF1* deletions represent a further genetic poor prognostic factor in Ph-positive ALL? *IKZF1* encodes a zinc finger protein required for lymphoid lineage differentiation, proliferation, and function.[26,27] The transcription factor Ikaros exerts its effects in development as a set of differentially spliced isoforms that contain two functionally distinct Kruppel-type zinc finger domains, one involved in DNA binding and the second involved in protein interactions.[26] Recently, findings by Mullighan et al[14] and our present study strongly demonstrate that intragenic deletions in the *IKZF1* gene are responsible for the generation of different aberrant isoforms. In this work, we correlated the occurrence of the *IKZF1* deletion with the clinical outcome to assess whether this genomic abnormality may be considered a

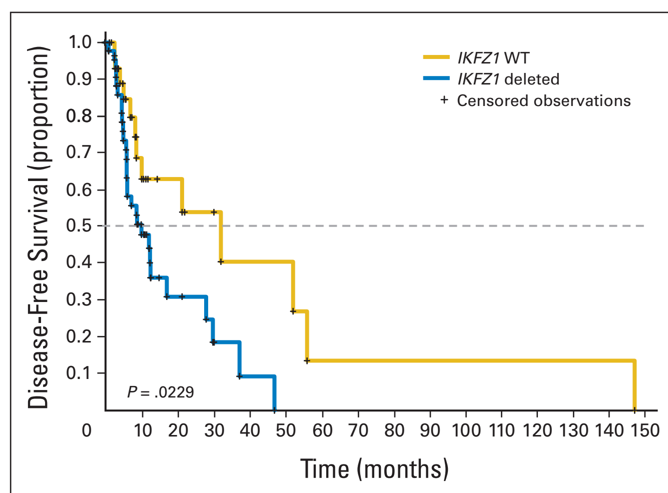

Fig 3. Disease-free survival of de novo–treated patients with Philadelphia chromosome–positive acute lymphoblastic leukemia treated with conventional or investigational therapy including tyrosine kinase inhibitor (imatinib or dasatinib) regimens and with *IKZF1* deletion compared with patients treated with the same protocols without *IKZF1* deletion (*IKZF1* wild type [WT]).

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prognostic marker. Most of the patients in our study have been treated with a TKI, such as imatinib, dasatinib, or another TKI (second- and third-generation TKIs), either as first-line therapy or as maintenance therapy. Even if the treatment with TKIs has increased the rate of patients obtaining a CHR and has prolonged OS and DFS in the majority of patients with Ph-positive ALL (including elderly patients), true resistance to TKIs can still occur after a few months of TKI therapy. The expression of non–DNA-binding Ikaros isoforms correlated with *BCR-ABL1* mRNA levels, as we have reported,[20] disease progression, relapse, and resistance to imatinib and dasatinib.

Overall, these new findings emphasize specific genetic differences between Ph-positive ALL subtypes and between individual patients and suggest that recurrent gene copy number losses affecting B-cell differentiation are universal in Ph-positive ALL. Because *IKZF1* deletions occur in more than 60% of patients with Ph-positive ALL at diagnosis and represent a poor prognostic marker, we suggest that this analysis should be considered as a routine screening assay for this aggressive *BCR-ABL1*–induced lymphoid malignancy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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