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Prognostic impact of kinase-activating fusions and IKZF1 deletions in pediatric high-risk B-lineage acute lymphoblastic leukemia

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Key Points

- Fifteen percent of NCI high-risk, Ph-negative, B-ALL patients harbored a kinase-activating fusion, and often associated with *IKZF1* deletion.
- *IKZF1* deletion represents an independent prognostic factor of poor outcomes, regardless of fusion-positivity.

Recurrent chromosomal rearrangements carry prognostic significance in pediatric B-lineage acute lymphoblastic leukemia (B-ALL). Recent genome-wide analyses identified a high-risk B-ALL subtype characterized by a diverse spectrum of genetic alterations activating kinases and cytokine receptor genes. This subtype is associated with a poor prognosis when treated with conventional chemotherapy but has demonstrated sensitivity to the relevant tyrosine kinase inhibitors. We sought to determine the frequency of kinase-activating fusions among National Cancer Institute (NCI) high-risk, Ph-negative, B-ALL patients enrolled on Dana-Farber Cancer Institute ALL Consortium Protocol 05-001 and to describe their associated clinical characteristics and outcomes. Among the 105 patients screened, 16 (15%) harbored an ABL-class fusion (*ETV6-ABL1*: n = 1; *FOXP1-ABL1*: n = 1; *SFPQ-ABL1*: n = 1; *ZC3HAV1-ABL2*: n = 1) or a fusion activating the JAK-STAT pathway (*P2RY8-CRLF2*: n = 8; *PAX5-JAK2*: n = 4). Sixty-nine percent of patients with an identified fusion had a concomitant *IKZF1* deletion (n = 11). In univariate analysis, fusion-positivity and *IKZF1* deletion were each associated with inferior event-free survival; *IKZF1* deletion retained statistical significance in multivariable analysis (hazard ratio, 2.64; $P = .019$). Our findings support therapy intensification for *IKZF1*-altered patients, irrespective of the presence of a kinase-activating fusion.

Introduction

Over the last several decades, cure rates for childhood acute lymphoblastic leukemia (ALL) have drastically improved, with overall survival now approaching 90%.[1] However, relapse still occurs in approximately 15% of patients, and long-term cure rates postrelapse remain poor, making ALL the second leading cause of cancer-related death in children.[2]

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Table 1. Patient characteristics by fusion status

		Kinase-activating fusion				
		No		Yes		
	N	N	%	N	%	*P*
Number screened	105	89	85	16	15	
Age at diagnosis						.003
<10 y	49	47	96	2	4	
≥10 y	56	42	75	14	25	
Median (range)	10.6 (1.3-18.0)	7.4 (1.3-17.1)		13.5 (1.8-18.0)		.016
Leukocyte count at diagnosis, $\times 10^9$/L						.38
<50	34	27	79	7	21	
≥50	71	62	87	9	13	
Median (range)	70.0 (1.3-726.3)	75.2 (1.3-726.3)		51.8 (5.5-363.8)		.43
Sex						.031
Female	46	43	93	3	7	
Male	59	46	87	13	22	
Ethnicity*						.014
Hispanic or Latino	24	17	71	7	29	
Non-Hispanic	74	68	92	6	8	
Ethnicity not known	7	4	57	3	43	
***IKZF1* deleted*†**						<.001
No	66	61	92	5	8	
Yes	28	17	61	11	39	
Cytogenetics						
Hyperdiploidy (51-65 chromosomes)	14	14	100	0	0	.12
ETV6-RUNX1	16	16	100	0	0	.12
KMT2A rearrangement	8	7	88	1	13	1.00
TCF3-PBX1	8	7	88	1	13	1.00
Achieve CR	96	82	85	14	15	.62
MRD*						.08
High	10	8	80	2	20	
Low	72	64	89	8	11	
Indeterminate	14	10	71	4	28	
Final risk						.45
High	81	70	86	14	17	
Very high	15	12	80	3	20	

CR, complete remission.
*The *P* value excludes unknowns.
†Ninety-four of the 105 were also screened for *IKZF1*.

Recent large-scale genome-wide studies have enabled the identification of actionable genomic alterations, providing the rationale to test targeted therapies in genomically defined patient subsets. In particular, *BCR-ABL1*-like ALL (or Ph-like ALL) constitutes a new high-risk B-lineage ALL (B-ALL) subgroup, comprising approximately 15% of children and adolescents with B-ALL.[3] Lymphoblasts from these patients display a gene expression signature similar to that of Philadelphia chromosome–positive (Ph^+) ALL but lack the canonical *BCR-ABL1* gene fusion.[4,5] Ph-like status is more common among National Cancer Institute (NCI) high-risk B-ALL patients and has been associated with inferior outcomes.[3,6] A distinguishing feature of Ph-like ALL is a heterogeneous spectrum of genomic alterations activating kinases and cytokine receptor genes that are amenable to inhibition with the relevant tyrosine kinase inhibitors (TKIs).[3] A high proportion of Ph-like ALL patients also exhibits *IKZF1* deletion, which has also been reported to be an independent predictor of adverse outcome in pediatric B-ALL.[7-9] The objectives of our study were to determine the frequency and prognostic significance of TKI-targetable kinase-activating fusions in NCI high-risk, Ph-negative B-ALL patients treated on the Dana-Farber Cancer Institute (DFCI) ALL Consortium protocol 05-001.

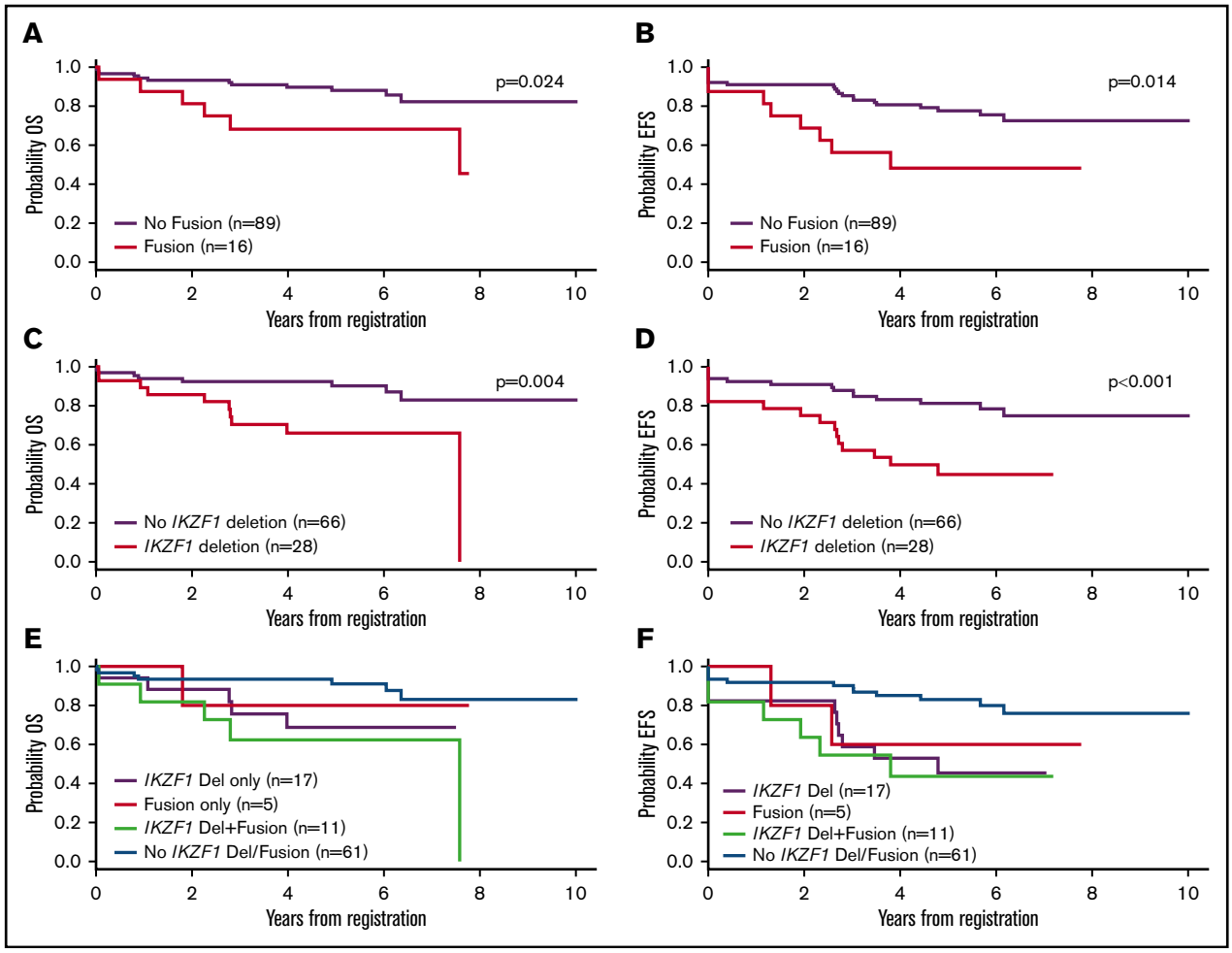

Figure 1. Overall and event-free survival of NCI high-risk, Ph-negative, B-ALL patients by fusion and *IKZF1* status. (A-B) The 5-year OS and EFS for patients with Fusion$^+$ patients were 68% (95% confidence interval [CI], 39% to 85%) and 48% (95% CI, 22% to 70%), respectively, in comparison with 88% (95% CI, 79% to 93%) and 78% (95% CI, 67% to 85%) for fusion-negative patients. (C-D) The 5-year OS and EFS for patients with *IKZF1* deletion were 66% (95% CI, 45% to 81%) and 45% (95% CI, 25% to 62%), respectively, in comparison with 90% (95% CI, 79% to 95%) and 81% (95% CI, 69% to 89%) for patients without *IKZF1* deletion. (E-F) The 5-year OS and EFS were 62% (95% CI, 28% to 84%) and 44% (95% CI, 15% to 70%), respectively, for patients with both fusion-positivity and *IKZF1* deletion in comparison with 91% (95% CI, 79% to 96%) ($P = .005$) and 83% (95% CI, 71% to 90%) ($P = .006$) for those with neither. Del, deletion.

Methods

Patients and samples

Between 2005 and 2011, 219 newly diagnosed NCI high-risk, Ph-negative, B-ALL patients, aged 1 to 18 years old, were enrolled on DFCI ALL Consortium protocol 05-001 (NCT00400946).[10] One hundred five of these patients had sufficient banked material to undergo kinase fusion testing by validated multiplex reverse transcriptase polymerase chain reaction (RT-PCR) assays, of whom 94 also had material available for assessment of *IKZF1* gene deletion status. Patients, their parents/guardians, or both provided informed consent for trial participation, banking, and future research prior to enrollment.

Therapy

Treatment on DFCI 05-001 has been previously described.[10] NCI high-risk, Ph-negative B-ALL patients with any of the following characteristics were treated in the very high risk group: *KMT2A* rearrangements, low hypodiploidy, and/or high end-induction minimal residual disease (MRD), defined as $\geq 10^{-3}$ (≥ 0.001) as assessed by real-time quantitative PCR analysis of patient-specific antigen receptor gene rearrangements. All other NCI high-risk, Ph-negative B-ALL patients were treated as high risk.

Kinase fusion and IKZF1 status detection

Diagnostic marrow samples were subject to multiplex PCR for identifying kinase fusions in Ph-like ALL, as has been previously described.[6] Each purified PCR product was sequenced individually using the reverse primer present in the amplification master mix and analyzed by using the NCBI database to identify the forward partner of the fusion. A singleplex PCR reaction was set up to confirm the identity of the positive RT-PCR result using the appropriate forward and reverse primers. *IKZF1* deletion status was assessed by multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA P202 IKZF1; MRC-Holland), as has been previously described.[7]

Table 2. Univariate and multivariable Cox proportional hazards models for event-free survival

	Univariate (n = 94)		Multivariable (n = 94)	
	HR (95% CI)	P	HR (95% CI)	P
Fusion (yes vs no)	2.66 (1.17-6.02)	.019	1.45 (0.58-3.61)	.43
WBC (≥50 vs <50 × 10^9/L)	1.64 (0.70-3.85)	.25	1.48 (0.63-3.50)	.25
IKZF1 deletion (yes vs no)	3.21 (1.55-6.68)	.002	2.73 (1.20-6.21)	.016

Table 3. Univariate and multivariable Cox proportional hazards models for disease-free survival

	Univariate (n = 72)		Multivariable (n = 72)	
	HR (95% CI)	P	HR (95% CI)	P
Fusion (yes vs no)	3.29 (1.26-8.59)	.015	0.46 (0.09-2.23)	.34
WBC (≥50 vs <50 × 10^9/L)	0.99 (0.39-2.48)	.98	1.26 (0.40-3.96)	.69
IKZF1 deletion (yes vs no)	3.27 (1.36-7.88)	.008	2.70 (0.90-8.14)	.087
MRD (high vs low)	3.30 (1.15-9.51)	.027	2.19 (0.72-6.66)	.17

Statistical analysis

Categorical and continuous patient characteristics were compared by fusion status with the Fisher's exact test and the Wilcoxon rank sum test, respectively. Event-free survival (EFS), overall survival (OS), and disease-free survival (DFS) were estimated with the Kaplan-Meier method and compared using a log-rank test. DFS was defined for those patients entering a complete remission. Univariate and multivariable Cox proportional hazards models of EFS and DFS were also constructed by considering fusion-positivity, *IKZF1* deletion, and diagnostic white blood cell count (WBC). DFS also considered end-induction MRD.

Results

Of the NCI high-risk, Ph-negative, B-ALL patients enrolled on DFCI 05-001, a higher proportion of patients screened for fusions were age <10 years (47% vs 20%; $P < .001$) and had WBC ≥50 × 10^9/L at diagnosis (68% vs 25%; $P < .001$) in comparison with those who were not screened. Among the 105 patients screened, 16 (15%) were found to harbor either an ABL-class fusion (*ETV6-ABL1*: n = 1; *FOXP1-ABL1*: n = 1; *SFPQ-ABL1*: n = 1; *ZC3HAV1-ABL2*: n = 1) or a fusion activating the JAK-STAT pathway (*P2RY8-CRLF2*: n = 8; *PAX5-JAK2*: n = 4). Of the 16 patients with an identified kinase fusion (Fusion$^+$), 11 (69%) had a concomitant *IKZF1* deletion. Features associated with fusion-positivity were age ≥10 years ($P = .003$), male sex ($P = .031$), Hispanic ethnicity ($P = .014$), and *IKZF1* deletion ($P < .001$) (Table 1). None of the Fusion$^+$ patients had favorable cytogenetics such as hyperdiploidy or *ETV6-RUNX1*. Fifty percent of Fusion$^+$ patients experienced an event (induction death: n = 1; induction failure: n = 1; relapse: n = 6) in comparison with 24% of patients without a kinase fusion. Fusion$^+$ patients had significantly inferior OS and EFS than did those without kinase fusions (Figure 1A-B). These findings are comparable to previous reports in terms of the frequency, genomic distribution, clinical characteristics, and outcomes of Ph-like ALL patients within this risk group.[3]

Twenty-eight of 94 patients (30%) assessed were found to harbor *IKZF1* deletions. Clinical features associated with the presence of the *IKZF1* deletion were male sex ($P = .045$) and Hispanic ethnicity ($P = .011$). As with fusion-positivity, the presence of *IKZF1* deletion was associated with inferior outcome (Figure 1C-D), as has been reported by others.[7-9] In univariate analysis, fusion-positivity (hazard ratio [HR], 2.66, $P = .019$) and *IKZF1* deletion (HR, 3.21, $P = .002$) were each significantly associated with inferior EFS (Table 2). In multivariable analysis, only *IKZF1* deletion retained statistical significance (HR, 2.64, $P = .016$). Univariately, fusion-positivity (HR, 3.29, $P = .015$), *IKZF1* deletion (HR, 3.27, $P = .008$), and high end-induction MRD (HR, 3.30, $P = .027$) were also significantly associated with inferior DFS (Table 3). None of the above variables retained significance in the multivariable analysis for DFS, although there was a trend toward inferiority for *IKZF1* deletion (HR, 2.70, $P = .087$). Additionally, in analyses comparing OS and EFS in patients with both *IKZF1* deletion and fusion-positivity, with one or neither of these alterations, patients with concomitant Fusion$^+$/*IKZF1* deletion ($P = .005$, $P = .006$) or *IKZF1* deletion alone ($P = .050$, $P = .003$) had significantly worse outcomes than did those with neither alteration (Figure 1E-F).

Discussion

Our study identified that 30% of NCI high-risk, Ph-negative B-ALL patients harbored an *IKZF1* gene deletion and 15% harbored a kinase-activating fusion, frequently in association with an *IKZF1* deletion. We demonstrated that the presence of a kinase-activating fusion was associated with an inferior outcome; however, *IKZF1* deletion status represents an independent predictor of adverse outcome in NCI high-risk B-ALL patients, regardless of fusion status. Although screening for fusion status may identify high-risk patients who may respond to targeted therapies, our data would suggest that screening for *IKZF1* deletions may enhance current risk classification strategies, identifying additional, kinase-negative patients who may benefit from intensified or novel therapies.

The inferior outcomes among fusion-positive patients in the DFCI cohort validate the need to find alternative therapeutic approaches for this patient population. These kinase fusions have been shown to be sensitive to the relevant TKIs in vitro and in patient-derived xenograft models.[3,11] A number of clinical trial groups, including our own, are now conducting prospective studies to identify patients with actionable kinase-activating fusions at diagnosis and determine whether combining the relevant TKI with a high-risk chemotherapy backbone will improve their outcomes.

Our study has several limitations. First, the frequency of fusion-positive patients in our cohort is underestimated because our RT-PCR assays are not suitable for detecting *IGH-CRLF2* and *EPOR* rearrangements. Alternative approaches, such as the Archer anchored multiplex PCR and transcriptome sequencing, may uncover more fusions than would our focused RT-PCR assay. Additional limitations of our study include the restriction to only NCI high-risk patients and retrospective testing of banked patient samples on the basis of specimen availability. These limitations are addressed in our current trial, DFCI ALL Consortium protocol 16-001, which utilizes a next-generation sequencing assay to identify

IKZF1 deletions and an unbiased RNA-sequencing approach to prospectively identify targetable kinase fusions in all newly diagnosed B-ALL patients, regardless of NCI risk group, to enhance risk classification and stratify therapy.

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Authorship

Contribution: T.H.T., M.H.H., M.L.L., and L.B.S. designed the study; T.H.T. and J.V.N. performed the kinase fusion screen; M.H.H. performed the *IKZF1* deletion screen; B.L.A., U.H.A., L.A.C., P.D.C., K.M.K., C.L., J.-M.L., B.M., M.A.S., J.J.G.W., L.B.S., and S.E.S. enrolled patients and collected data; E.S. and S.C.R. assisted with the kinase fusion screen; T.M.B., K.E.S., and D.S.N. did the statistical analysis; T.H.T., T.M.B., K.E.S., M.H.H., M.L.L., and L.B.S. analyzed the data and prepared the manuscript; all authors revised and approved the manuscript.

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