#### TO THE EDITOR:

# *IKZF1* alterations predict poor prognosis in adult and pediatric T-ALL

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T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are aggressive neoplasms that result from the proliferation of T-lymphoid progenitors blocked at thymic stages of differentiation. They account for 15% and 25% of pediatric and adult ALLs, respectively. T-ALL/T-LBL are associated with a wide range of acquired genetic abnormalities that contribute to developmental arrest and abnormal proliferation.<sup>12</sup> Although intensive treatment protocols have markedly improved the outcomes of children with T-ALL, cure rates remain below 60% for adults and 85% for children.<sup>3-5</sup> The prognosis is particularly poor in relapsing patients, highlighting an urgent need for risk stratification factors at diagnosis.<sup>6,7</sup>

The IKAROS transcription factor (encoded by the *IKZF1* gene on chromosome 7p12.2) is a member of the zinc finger family of DNA-binding proteins that acts as a critical regulator of hematopoiesis and lymphoid differentiation.<sup>8</sup> *IKZF1* is recurrently affected by various genetic alterations in B-cell acute lymphoblastic leukemia (B-ALL). Genomic alterations in *IKZF1* are found in ~15% of childhood B-ALL cases and in 40% of adult B-ALL cases, with a higher incidence in poor prognosis cases, including *BCR-ABL1* (70%) or *BCR-ABL1*-like (40%) B-ALL.<sup>9,10</sup> Of note, the *IKZF1* alteration consistently exhibited its poor prognostic impact in B-ALL, and clinical trials increasingly integrate *IKZF1* gene status in risk stratification algorithms.<sup>11,12</sup>

In contrast, both the incidence and prognostic influence of *IKZF1* alterations in T-ALL/T-LBL are poorly characterized.<sup>13</sup> To specify the role of *IKZF1* alterations in T-ALL/T-LBL, we conducted a comprehensive analysis using pan-exon deep sequencing of 1260 adult and pediatric T-ALL/T-LBL patients (supplemental Figure 2, available on the *Blood* Web site), including 980 T-ALL cases and 280 T-LBL cases. Diagnostic DNA samples were analyzed by using an 80-gene pan-exon capture-panel (details included in the supplemental Methods). *IKZF1<sup>Alt</sup>* screening was performed by computational approaches previously described for the detection of copy number variants from next-generation sequencing data.<sup>14</sup> *IKZF1* deletions were confirmed with multiplex ligation-dependent probe

amplification (MLPA) analysis and/or microarray-based comparative genomic hybridization (array CGH). Patient protocols and clinical trials,<sup>3,15,16</sup> immunophenotypic and molecular characterization of T-ALL and T-LBL samples, minimal residual disease (MRD) assessment, gene mutation screening, array CGH, MLPA, statistical analysis, and additional details are included in the supplemental Methods.

IKZF1 mutations were identified in 42 cases, including 33 (3.4%) of 980 T-ALL cases and 9 (3.2%) of 280 T-LBL cases (Figure 1A). The majority of mutations were missense (24 of 42 [60%]) within a mutational hotspot in exon 5 affecting amino acid p.N159 (N159S/ T) located in the DNA-binding domain. Interestingly, this mutation was recently described in a new combined immunodeficiency syndrome with potential risk of T-ALL predisposition.<sup>17,18</sup> We also detected frameshift or nonsense mutations (17 of 42 [40%]) affecting exons 3-8 and predicted to truncate the protein before the C-terminal dimerization domain, resulting in haploinsufficiency. IKZF1 deletions were detected in 40 cases (3%) including 37 (3.8%) of 980 and 3 (1.5%) of 280 in T-ALL and T-LBL, respectively (Figure 1B). All were confirmed by MLPA and/or array CGH (supplemental Figure 2; supplemental Figure 3A-B). Of note, most cases (30 of 40 [75%]) harbored pan-genic deletions (exons 1-8), leading to haploinsufficiency; only 10% (4 of 40) intragenic deletions (exons 4-7), predicted to induce a dominant-negative effect, were observed. This suggests that, in contrast to B-cell precursor-ALL (BCP-ALL),12 the main consequences of IKZF1 deletions in T-ALL would be haploinsufficiency rather than a dominant-negative effect. IKZF1 deletions and mutations were mutually exclusive, and no biallelic inactivation of IKZF1 was observed, suggesting that residual IKZF1 activity may be required for T-lineage leukemogenesis. Overall, IKZF1<sup>Alt</sup> was identified in 82 (6.5%) of 1260 T-ALL/T-LBL cases (7.1% T-ALL and 4.3% T-LBL) and were more frequent within adult T-ALL/ T-LBL cases compared with pediatric cases (54 of 699 adult T-ALL/T-LBL compared with 28 of 561 pediatric cases; P = .05) (supplemental Figure 2). The oncoplot highlighting the main comutations observed and mutations within individual *IKZF1*<sup>Alt</sup> cases are reported in supplemental Table 3 and supplemental Figure 4.

Figure 1. IKZF1 mutational and deletion patterns according to patient occurrence. (A) Gene map describing IKZF1 intragenic mutational patterns according to patient occurrence. (B) Gene map describing IKZF1 deletion patterns according to patient occurrence. (C) Genetic profiles of IKZF1<sup>Alt</sup> T-ALL in the FRALLE and GRAALL 03-05 protocols. Comparison of mutational profiles according to pathways between IKZF1<sup>Alt</sup> T-ALLs (n = 26) and  $IKZF1^{GL}$  T-ALLs (n = 450), with a focus on alterations found in at least 5% of the whole cohort. Percent frequencies in each group are indicated. Genes are grouped according to functional categories. OS (D) and cumulative incidence of relapse (E) in FRALLE and GRAALL treated patients. \*P < .05. IL7R, interleukin-7 receptor; PI3K-Akt, phosphatidylinositol 3-kinase/protein kinase B.



We then investigated the clinical characteristics linked to  $IKZF1^{A/t}$  in a subset of 476 patients, including 215 adults enrolled in the GRAALL-2003/05 trials and 261 children enrolled in the

FRALLE-2000 trial (GRAALL-2003, #NCT00222027; GRAALL-2005, #NCT00327678) (Table 1; supplemental Methods). Diagnostic peripheral blood or bone marrow samples from 1258 adults and

## Table 1. Clinico-biological and outcome characteristics of adult and pediatric T-ALL cases (GRAALL and FRALLE protocols) according to *IKZF1* status

	No. (%) of patients or median (95% CI)							
Characteristic	IK	IKZF1 <sup>Alt</sup> IKZF1 <sup>GL</sup>		F1 <sup>GL</sup>			otal	Р
Total	26	(5.5%)	450	(94.5%)	4	76	(100%)	
Clinical subsets analyzed Male sex Age, median (range), y Median WBC (range), ×10°/L CNS involvement	21 23.5 74.1 4/26	(81%) (1.1-59.1) (2.8-641) (15%)	339 15.2 63.4 47/448	(75%) (1.2-59) (0.3-980) (11%)	3 1! 6: 51/	60 5.4 3.8 474	(75%) (1.1-59.1) (0.3-980) (11%)	.6 .1 .3 .5
ETP classification	4/16	(25%)	51/291	(18%)	55/	307	(18%)	.5
<b>TCR status</b> Immature (IM0/δ/γ) Cortical (IMB, preαβ) Mature TCRαβ Mature TCRγδ	17 8 5 3 1	(47%) (29%) (18%) (6%)	295 60 154 42 39	(20%) (52%) (14%) (13%)	312 68 159 45 40		(22%) (51%) (14%) (13%)	.02* .08 .7 .7
Oncogenetics TLX1 TLX3 SIL-TAL1 CALM-AF10 None of above HOXA positive	24 1 2 1 16 4/21	(4%) (17%) (8%) (4%) (67%) (19%)	390 53 68 55 12 202 74/315	(14%) (17%) (14%) (3%) (52%) (23%)	414 54 72 57 13 218 78/336		(13%) (17%) (14%) (3%) (53%) (24%)	.3 .9 .6 .5 .2 .8
N/F-R/P classifier High-risk classifier NOTCH1/FBXW7 <sup>mut</sup> K/N-RAS <sup>mut</sup> PTEN <sup>altered</sup>	26 15 19 8 2	(58%) (73%) (31%) (8%)	450 198 302 41 80	(44%) (67%) (9%) (18%)	476 213 321 49 82		(45%) (67%) (10%) (17%)	.2 .7 .003* .3
Treatment response CR Prednisone response MRD1 $\geq 10^{-4}$ HSCT	24/26 14/26 12/18 3/24	(92%) (54%) (67%) (13%)	416/450 245/441 111/322 93/416	(92%) (56%) (34%) (22%)	440/476 259/467 123/340 96/440		(92%) (55%) (36%) (22%)	1 1 .01* .3
Outcome 5 y CIR (95% CI) 5 y OS (95% CI)	50% 37%	(32-71) (19-55)	28% 73%	(24-32) (69-77)	32) 29 '-77) 71		(25-33) (67-75)	.01* <.001*
	Univariate analysis				Multivariate analysis			
CIR Age* Log(WBC)* Prednisone response 4-gene classifier† <i>IKZF1<sup>Alt</sup></i> OS Age* Log(WBC)* Prednisone response 4-gene classifier†	1.01 1.62 0.67 2.78 2.12 1.03 1.99 0.54 2.93	0.98-1.0 1.20-2.18 0.47-0.95 1.94-3.99 1.17-3.86 1.01-1.05 1.48-2.67 0.38-0.76 2.06-4.17	.57 .02 .026 <.001 .013 .001 <.001 <.001 <.001	1.0 2.0 2.1 1.0 2.0 2.1	- 50 22 59 15 04 04 27 9 38	1.18-2.17 0.63-1.34 1.86-3.88 1.18-3.91 1.02-1.06 1.49-2.79 0.55-1.15 2.00-4.16		.003 .66 <.001 .012 <.001 <.001 .21 <.001

MRD1 corresponds to MRD evaluation after induction and was performed by allele-specific oligonucleotides polymerase chain reaction. T-cell receptor (TCR) status and oncogenic evaluations were performed as described in the supplemental Methods. CIR, cumulative incidence of relapse; CNS, central nervous system; CR, complete remission; ETP, early thymic precursor; N/F-R/P classifier, NOTCH1/FBXW7-RAS/PTEN classifier (as previously described); HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; SHR, specific hazard ratio; WBC, white blood cell. Univariate and multivariate analyses of cumulative incidence of relapse and OS: \*Continuous variables; <sup>†</sup>Presence of RAS/PTEN alteration and/or absence of NOTCH1/FBXW7 mutations.

children with T-ALL or T-LBL were analyzed after informed consent was obtained at diagnosis according to the Declaration of Helsinki. The incidence of IKZF1<sup>Alt</sup> in this cohort was 5.5% (26 of 476), including 16 deletions and 10 mutations. IKZF1<sup>Alt</sup> were observed in 7% of adults and 4.2% of children (P = .2), with a median age slightly higher in *IKZF1*<sup>Alt</sup> cases (23.5 years vs 15.2 years; P = .1). IKZF1<sup>Alt</sup> was associated with an immature immunophenotype (47% vs 20%; P = .02). In line with this finding, *IKZF1*<sup>Alt</sup> correlated positively with abnormalities known to be associated with an immature phenotype, including K/N-RAS mutations (31% vs 9%; P = .003), EZH2 (5 of 26 [20%] vs 27 of 450 [6%]; P = .02), ASXL1 (4 of 26 [15%] vs 15 of 450 [3%]; P = .02), ETV6 (4 of 26 [15%] vs 13 of 450 [3%]; P = .01), and DNMT3A (5 of 26 [20%] vs 17 of 450 [4%]; P = .045) mutations (Figure 1C). Conversely, IKZF1<sup>Alt</sup> were virtually exclusive, with SIL-TAL1<sup>+</sup> and PTEN altered cases known to be associated with a mature T-cell receptor- $\alpha\beta$  lineage.

In BCP-ALL, *IKZF1*<sup>Alt</sup> are enriched in the high-risk subgroup of *Ph*<sup>+</sup> BCP-ALL and recently characterized by the presence of a gene expression profile similar to *Ph*<sup>+</sup> ALL but lacking the canonical *BCR-ABL1* fusion, therefore named *BCR-ABL1-like* or *Ph*<sup>+</sup>-*like* ALL.<sup>9,19</sup> Importantly, *Ph*<sup>+</sup>-*like* signature is virtually absent in T-ALL.<sup>20</sup> In contrast, here we observed that *IKZF1*<sup>Alt</sup> are associated with epigenetic mutations/deletions.

Interestingly, *IKZF1*-deficient mice develop T-cell malignancy with high penetrance, highlighting the suppressor function for IKAROS in T-cell lineage.<sup>21</sup> Furthermore, in murine T-cell leukemogenesis, IKAROS directly cooperates with NOTCH1 activation to promote leukemia.<sup>22,23</sup> This crosstalk between NOTCH1 and IKAROS could explain the discrepancy observed concerning the pattern of *IKZF1*<sup>A/t</sup> in T-ALL vs *Ph*<sup>+</sup>-*like* BCP-ALL, and it led us to suspect a specific oncogenic mechanism associated with *IKZF1*<sup>A/t</sup> in T-ALL requiring further investigations.

IKZF1<sup>Alt</sup> cases did not differ significantly with regard to sex, white blood cell count, central nervous system involvement, and prednisone response (Table 1). Although IKZF1<sup>Alt</sup> did not affect the complete remission rate, patients with IKZF1<sup>Alt</sup> were more likely to have a positive postinduction MRD (10<sup>-4</sup> threshold, 67% vs 34%; P = .01). Patients with *IKZF1*<sup>A/t</sup> had an inferior outcome compared vs those with IKZF1GL, with a higher cumulative incidence of relapse (5-year cumulative incidence of relapse, 50% vs 28%; specific hazard ratio, 2.12; 95% confidence interval [CI], 1.17-3.86) and a shorter overall survival (OS) (5-year OS, 37% vs 73%; hazard ratio, 2.94; 95% CI, 1.74-4.96) (Figure 1D-E). This prognostic impact was observed in both pediatric and adult cohorts (supplemental Figure 5A-D). Of note, the 10 IKZF1-mutated and 16 IKZF1deleted cases exhibited comparable clinico-biological features and were associated with worse prognosis compared with IKZF1<sup>GL</sup> cases (supplemental Table 4; supplemental Figure 6). In multivariate analysis, considering variables associated with OS in univariate analyses as covariates, IKZF1<sup>Alt</sup> remained significantly associated with a shorter OS, even after inclusion of postinduction MRD in the model (supplemental Table 5). It is noteworthy that the prognostic impact of *IKZF1<sup>Alt</sup>* status was also observed after adjustment on the 4-gene NOTCH1/ FBXW7/RAS/PTEN classifier and postinduction MRD, which identified poor prognosis patients in both GRAALL and FRALLE trials.3,4

In conclusion, we describe *IKZF1<sup>A/t</sup>* among 1260 children and adults with immature T-ALL/T-LBL and define for the first time its frequency and, importantly, its poor outcome in T-ALL in multivariate models. *IKZF1<sup>A/t</sup>* should be considered as a significant prognosis marker in addition to MRD and the 4-gene oncogenetic classifier to predict poor outcomes in T-ALL.

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## Authorship

Contribution: N.B., V.A., and M.S. conceived the study and oversaw the project; M.S., M.-E.D., L.L., E.L., C.G., N.G., J.-M.C., I.A., V.G., N.I., H.D., A.B., A.P., and N.B. provided study materials or patients; M.S., L.L., E.M., and V.A. performed molecular analyses; M.S., L.L., and V.A. collected and assembled data; N.B. and M.S. performed statistical analysis; M.S., L.L., V.A., and N.B. analyzed and interpreted data; M.S., N.B., E.M., and V.A. wrote the manuscript; and all authors approved the manuscript.

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### Footnotes

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#### TO THE EDITOR:

# Early assessment of clofarabine effectiveness based on measurable residual disease, including AML stem cells

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Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and is characterized by heterogeneity in cytogenetics and molecular aberrations.<sup>1</sup> While insights in the pathogenesis and clonal landscape of AML has increased, the backbone of induction therapy has been the same for many years. In 1973, "7 +3" cytarabine plus anthracycline chemotherapy was first described,2 achieving a 5-year overall survival (OS) of  $\sim$ 40% to 50% in young patients<sup>3</sup> and 20% to 30% in elderly patients.<sup>4</sup> Several new (targeted) agents have been recently approved for AML,<sup>5</sup> which look promising in a subgroup of patients,<sup>6</sup> and some (untargeted) chemotherapeutic agents have been introduced. Clofarabine (2chloro-2'-fluoro-deoxy-9-β-d-arabinofuranosyladenine; a secondgeneration purine nucleoside analog), has shown potential benefit in young<sup>7</sup> and older<sup>8</sup> AML patients. However, as clofarabine was associated with a risk of severe complications in some studies9,10 but well tolerated in others,<sup>11,12</sup> further evaluation in dosing and scheduling was warranted. In a large phase 3 study of the

Dutch-Belgian Hemato-Oncology Cooperative Group–Swiss Group for Clinical Cancer Research, with  $\geq$ 800 patients enrolled, a significant favorable effect of clofarabine (added to idarubicin and Ara-C) was seen in the European LeukemiaNet (ELN) intermediate-I prognostic risk subgroup<sup>13</sup> (event-free survival, 26% ± 4% vs 40% ± 5%; Cox *P* = .002; OS, 29% ± 5% vs 50% ± 6%; Cox *P* < .001).<sup>10</sup>

One of the secondary objectives of the study was the assessment of efficacy according to measurable residual disease (MRD). Multiparameter flow cytometry (MFC)-MRD identifies leukemic cells, which can be distinguished from normal cells based on the presence of leukemia-associated immunophenotypes.<sup>14</sup> In addition, specific antibody panels allowed MFC assessment of leukemic stem cells (LSCs).<sup>15</sup> Samples for MFC-(LSC-)MRD detection were available for a subset of patients, and the time point of MFC-MRD assessment after 2 cycles of treatment was used for further analysis (median, 82 days after start of therapy; range,