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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.[1,2] 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.[3-5] The prognosis is particularly poor in relapsing patients, highlighting an urgent need for risk stratification factors at diagnosis.[6,7]

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.[8] *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.[9,10] 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.[11,12]

In contrast, both the incidence and prognostic influence of *IKZF1* alterations in T-ALL/T-LBL are poorly characterized.[13] 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). $IKZF1^{Alt}$ screening was performed by computational approaches previously described for the detection of copy number variants from next-generation sequencing data.[14] *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,[3,15,16] 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.[17,18] 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, $IKZF1^{Alt}$ 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 $IKZF1^{Alt}$ cases are reported in supplemental Table 3 and supplemental Figure 4.

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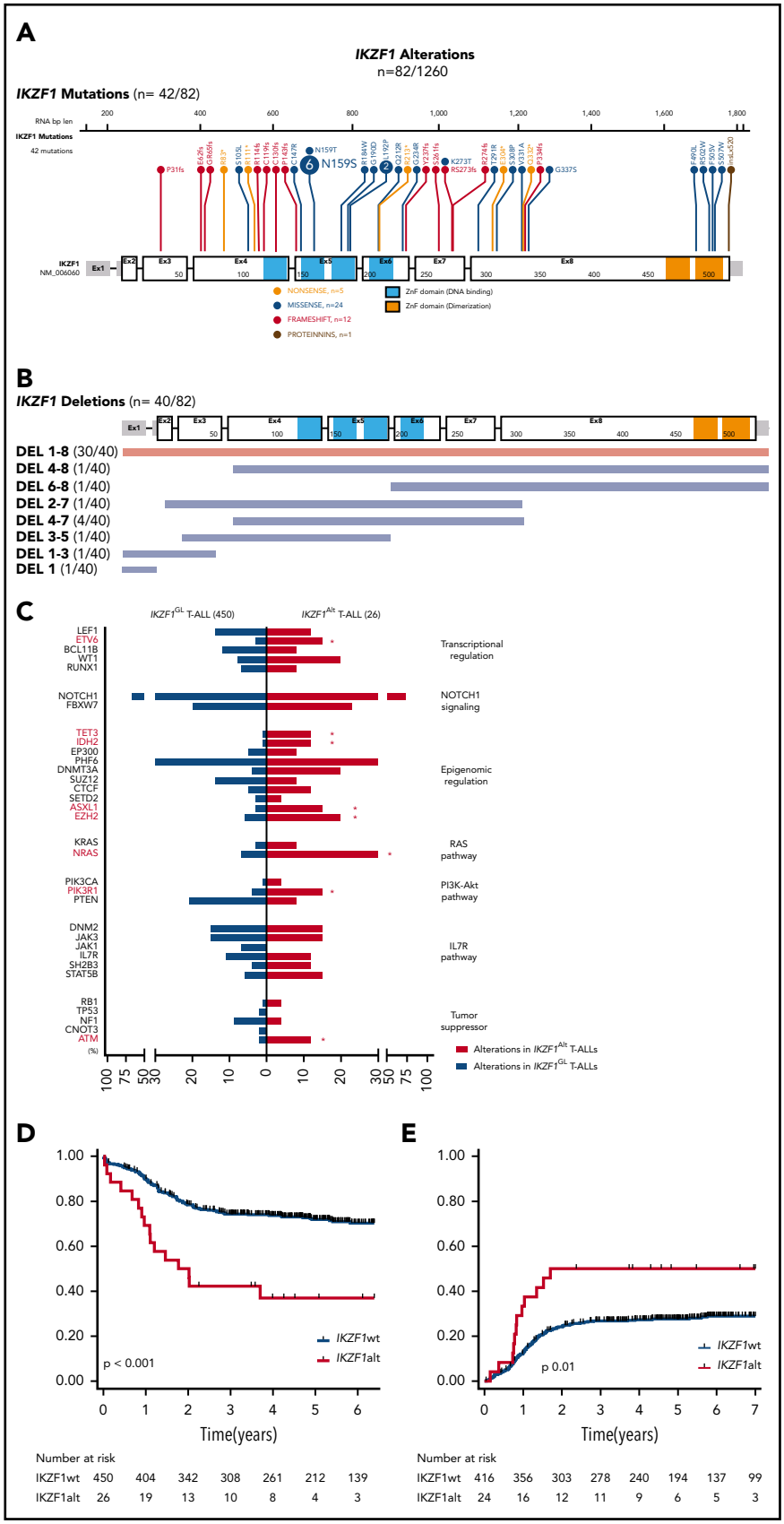

Figure 1. *IKZF1* mutational and deletion patterns according to patient occurrence. (A) Gene map describing *IKZF1* intragenic mutational patterns according to patient occurrence. (B) Gene map describing *IKZF1* deletion patterns according to patient occurrence. (C) Genetic profiles of $IKZF1^{Alt}$ T-ALL in the FRALLE and GRAALL 03-05 protocols. Comparison of mutational profiles according to pathways between $IKZF1^{Alt}$ 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 $IKZF1^{Alt}$ 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

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

Characteristic	No. (%) of patients or median (95% CI)						*P*
	IKZF1^{Alt}		*IKZF1*^{GL}		Total		
Total	26	(5.5%)	450	(94.5%)	476	(100%)	
Clinical subsets analyzed							
Male sex	21	(81%)	339	(75%)	360	(75%)	.6
Age, median (range), y	23.5	(1.1-59.1)	15.2	(1.2-59)	15.4	(1.1-59.1)	.1
Median WBC (range), $\times 10^9$/L	74.1	(2.8-641)	63.4	(0.3-980)	63.8	(0.3-980)	.3
CNS involvement	4/26	(15%)	47/448	(11%)	51/474	(11%)	.5
ETP classification	4/16	(25%)	51/291	(18%)	55/307	(18%)	.5
TCR status	17		295		312		
Immature (IM0/δ/γ)	8	(47%)	60	(20%)	68	(22%)	.02*
Cortical (IMB, preαβ)	5	(29%)	154	(52%)	159	(51%)	.08
Mature TCRαβ	3	(18%)	42	(14%)	45	(14%)	.7
Mature TCRγδ	1	(6%)	39	(13%)	40	(13%)	.7
Oncogenetics	24		390		414		
TLX1	1	(4%)	53	(14%)	54	(13%)	.3
TLX3	4	(17%)	68	(17%)	72	(17%)	.9
SIL-TAL1	2	(8%)	55	(14%)	57	(14%)	.6
CALM-AF10	1	(4%)	12	(3%)	13	(3%)	.5
None of above	16	(67%)	202	(52%)	218	(53%)	.2
HOXA positive	4/21	(19%)	74/315	(23%)	78/336	(24%)	.8
***N/F-R/P* classifier**	26		450		476		
High-risk classifier	15	(58%)	198	(44%)	213	(45%)	.2
NOTCH1/FBXW7^{mut}	19	(73%)	302	(67%)	321	(67%)	.7
K/N-RAS^{mut}	8	(31%)	41	(9%)	49	(10%)	.003*
PTEN^{altered}	2	(8%)	80	(18%)	82	(17%)	.3
Treatment response							
CR	24/26	(92%)	416/450	(92%)	440/476	(92%)	1
Prednisone response	14/26	(54%)	245/441	(56%)	259/467	(55%)	1
MRD1 $\geq 10^{-4}$	12/18	(67%)	111/322	(34%)	123/340	(36%)	.01*
HSCT	3/24	(13%)	93/416	(22%)	96/440	(22%)	.3
Outcome							
5 y CIR (95% CI)	50%	(32-71)	28%	(24-32)	29%	(25-33)	.01*
5 y OS (95% CI)	37%	(19-55)	73%	(69-77)	71%	(67-75)	<.001*

	Univariate analysis			Multivariate analysis		
	SHR	95% CI	*P*	SHR	95% CI	*P*
CIR						
Age*	1.01	0.98-1.0	.57	—	—	—
Log(WBC)*	1.62	1.20-2.18	.02	1.60	1.18-2.17	.003
Prednisone response	0.67	0.47-0.95	.026	0.92	0.63-1.34	.66
4-gene classifier†	2.78	1.94-3.99	<.001	2.69	1.86-3.88	<.001
IKZF1^{Alt}	2.12	1.17-3.86	.013	2.15	1.18-3.91	.012
OS						
Age*	1.03	1.01-1.05	.001	1.04	1.02-1.06	<.001
Log(WBC)*	1.99	1.48-2.67	<.001	2.04	1.49-2.79	<.001
Prednisone response	0.54	0.38-0.76	<.001	0.79	0.55-1.15	.21
4-gene classifier†	2.93	2.06-4.17	<.001	2.88	2.00-4.16	<.001
IKZF1^{Alt}	2.94	1.74-4.96	<.001	2.80	1.61-4.88	<.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; †Presence of *RAS/PTEN* alteration and/or absence of *NOTCH1/FBXW7* mutations.

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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*^{Alt} in this cohort was 5.5% (26 of 476), including 16 deletions and 10 mutations. *IKZF1*^{Alt} were observed in 7% of adults and 4.2% of children ($P = .2$), with a median age slightly higher in *IKZF1*^{Alt} cases (23.5 years vs 15.2 years; $P = .1$). *IKZF1*^{Alt} was associated with an immature immunophenotype (47% vs 20%; $P = .02$). In line with this finding, *IKZF1*^{Alt} 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*^{Alt} were virtually exclusive, with *SIL-TAL1*$^{+}$ and *PTEN* altered cases known to be associated with a mature T-cell receptor-αβ lineage.

In BCP-ALL, *IKZF1*^{Alt} are enriched in the high-risk subgroup of *Ph*$^{+}$ BCP-ALL and recently characterized by the presence of a gene expression profile similar to *Ph*$^{+}$ ALL but lacking the canonical *BCR-ABL1* fusion, therefore named *BCR-ABL1-like* or *Ph*$^{+}$*-like* ALL.[9,19] Importantly, *Ph*$^{+}$*-like* signature is virtually absent in T-ALL.[20] In contrast, here we observed that *IKZF1*^{Alt} 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.[21] Furthermore, in murine T-cell leukemogenesis, IKAROS directly cooperates with NOTCH1 activation to promote leukemia.[22,23] This crosstalk between NOTCH1 and IKAROS could explain the discrepancy observed concerning the pattern of *IKZF1*^{Alt} in T-ALL vs *Ph*$^{+}$*-like* BCP-ALL, and it led us to suspect a specific oncogenic mechanism associated with *IKZF1*^{Alt} in T-ALL requiring further investigations.

IKZF1^{Alt} cases did not differ significantly with regard to sex, white blood cell count, central nervous system involvement, and prednisone response (Table 1). Although *IKZF1*^{Alt} did not affect the complete remission rate, patients with *IKZF1*^{Alt} were more likely to have a positive postinduction MRD (10^{-4} threshold, 67% vs 34%; $P = .01$). Patients with *IKZF1*^{Alt} had an inferior outcome compared vs those with *IKZF1*^{GL}, 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 *IKZF1*-deleted cases exhibited comparable clinico-biological features and were associated with worse prognosis compared with *IKZF1*^{GL} cases (supplemental Table 4; supplemental Figure 6). In multivariate analysis, considering variables associated with OS in univariate analyses as covariates, *IKZF1*^{Alt} 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*^{Alt} 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*^{Alt} 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*^{Alt} 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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E-mail the corresponding author for original data.

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REFERENCES

1. Van Vlierberghe P, Ferrando A. The molecular basis of T cell acute lymphoblastic leukemia. *J Clin Invest*. 2012;122(10):3398-3406.

2. Girardi T, Vicente C, Cools J, De Keersmaecker K. The genetics and molecular biology of T-ALL. *Blood*. 2017;129(9):1113-1123.

3. Petit A, Trinquand A, Chevret S, et al; French Acute Lymphoblastic Leukemia Study Group (FRALLE). Oncogenetic mutations combined with MRD improve outcome prediction in pediatric T-cell acute lymphoblastic leukemia. *Blood*. 2018;131(3):289-300.

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4. Trinquand A, Tanguy-Schmidt A, Ben Abdelali R, et al. Toward a NOTCH1/FBXW7/RAS/PTEN-based oncogenetic risk classification of adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study. *J Clin Oncol*. 2013;31(34):4333-4342.

5. Gökbuget N, Kneba M, Raff T, et al; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. *Blood*. 2012;120(9):1868-1876.

6. Gökbuget N, Stanze D, Beck J, et al; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. *Blood*. 2012;120(10):2032-2041.

7. Desjonquères A, Chevallier P, Thomas X, et al. Acute lymphoblastic leukemia relapsing after first-line pediatric-inspired therapy: a retrospective GRAALL study. *Blood Cancer J*. 2016;6(12):e504.

8. Georgopoulos K, Bigby M, Wang JH, et al. The Ikaros gene is required for the development of all lymphoid lineages. *Cell*. 1994;79(1):143-156.

9. Mullighan CG, Su X, Zhang J, et al; Children's Oncology Group. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med*. 2009;360(5):470-480.

10. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature*. 2007;446(7137):758-764.

11. Stanulla M, Cavé H, Moorman AV. IKZF1 deletions in pediatric acute lymphoblastic leukemia: still a poor prognostic marker? *Blood*. 2020;135(4):252-260.

12. Vairy S, Tran TH. IKZF1 alterations in acute lymphoblastic leukemia: the good, the bad and the ugly. *Blood Rev*. 2020;44:100677.

13. Marçais A, Jeannet R, Hernandez L, et al. Genetic inactivation of Ikaros is a rare event in human T-ALL. *Leuk Res*. 2010;34(4):426-429.

14. Nord AS, Lee M, King MC, Walsh T. Accurate and exact CNV identification from targeted high-throughput sequence data. *BMC Genomics*. 2011;12(1):184.

15. Huguet F, Leguay T, Raffoux E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study [published correction appears in *J Clin Oncol*. 2009;27(15):2574]. *J Clin Oncol*. 2009;27(6):911-918.

16. Huguet F, Chevret S, Leguay T, et al; Group of Research on Adult ALL (GRAALL). Intensified therapy of acute lymphoblastic leukemia in adults: report of the Randomized GRAALL-2005 Clinical Trial. *J Clin Oncol*. 2018;36(24):2514-2523.

17. Boutboul D, Kuehn HS, Van de Wyngaert Z, et al. Dominant-negative IKZF1 mutations cause a T, B, and myeloid cell combined immunodeficiency. *J Clin Invest*. 2018;128(7):3071-3087.

18. Yoshida N, Sakaguchi H, Muramatsu H, et al. Germline IKAROS mutation associated with primary immunodeficiency that progressed to T-cell acute lymphoblastic leukemia. *Leukemia*. 2017;31(5):1221-1223.

19. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *Lancet Oncol*. 2009;10(2):125-134.

20. van der Veer A, Waanders E, Pieters R, et al. Independent prognostic value of BCR-ABL1-like signature and IKZF1 deletion, but not high CRLF2 expression, in children with B-cell precursor ALL. *Blood*. 2013;122(15):2622-2629.

21. Marke R, van Leeuwen FN, Scheijen B. The many faces of IKZF1 in B-cell precursor acute lymphoblastic leukemia. *Haematologica*. 2018;103(4):565-574.

22. Mantha S, Ward M, McCafferty J, et al. Activating Notch1 mutations are an early event in T-cell malignancy of Ikaros point mutant Plastic/+ mice. *Leuk Res*. 2007;31(3):321-327.

23. Kleinmann E, Geimer Le Lay AS, Sellars M, Kastner P, Chan S. Ikaros represses the transcriptional response to Notch signaling in T-cell development. *Mol Cell Biol*. 2008;28(24):7465-7475.

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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.[1] 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 ~40% to 50% in young patients[3] and 20% to 30% in elderly patients.[4] Several new (targeted) agents have been recently approved for AML,[5] which look promising in a subgroup of patients,[6] and some (untargeted) chemotherapeutic agents have been introduced. Clofarabine (2-chloro-2′-fluoro-deoxy-9-β-*d*-arabinofuranosyladenine; a second-generation purine nucleoside analog), has shown potential benefit in young[7] and older[8] AML patients. However, as clofarabine was associated with a risk of severe complications in some studies[9,10] but well tolerated in others,[11,12] 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 ≥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[13] (event-free survival, 26% ± 4% vs 40% ± 5%; Cox $P = .002$; OS, 29% ± 5% vs 50% ± 6%; Cox $P < .001$).[10]

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.[14] In addition, specific antibody panels allowed MFC assessment of leukemic stem cells (LSCs).[15] 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,