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

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Abstract:

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***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 which 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/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 which acts as a critical regulator of hematopoiesis and lymphoid differentiation.⁸ *IKZF1* is recurrently affected by various genetic alteration in B-cell acute lymphoblastic leukemia (B-ALL). Genomic alterations in *IKZF1* are found in about 15% of childhood and 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, *IKZF1* alteration consistently demonstrated 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/LBL are poorly characterized.¹³ To specify the role of *IKZF1* alterations in T-ALL/LBL we conducted a comprehensive analysis using pan-exon deep sequencing of 1260 adult and pediatric T-ALL/LBL patients (Flow chart, supplemental Figure 2) including 980 T-ALL and 280 T-LBL. Diagnostic DNA samples were analyzed using an 80-gene pan-exon capture-panel (details included in supplemental Methods). *IKZF1*^{Alt} screening was performed by computational approaches previously described for the detection of copy number variants (CNVs) 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 (CGHarray). 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 supplemental Methods.

IKZF1 mutations were identified in 42 cases including 33/980 (3.4%) and 9/280 (3.2%) of T-ALL and T-LBL respectively (Figure 1A). The majority of mutations were missense (24/42, 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 (17/42, 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 37/980 (3.8%) and 3/280 (1.5%) in T-ALL and T-LBL respectively (Figure 1B). All were confirmed by MLPA and/or CGH-array (supplemental Figure 2 and supplemental Figure 3A-B). Of note, most cases (30/40, 75%) harbored pan-genic deletions (exons 1-8) leading to haploinsufficiency and only 10% (4/40) intragenic deletions (exons 4-7), predicted to induce a dominant-negative effect, were observed. This suggests that, in contrast to BCP-ALL¹², the main consequences of *IKZF1* deletions in T-ALL would be haplo-insufficiency 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 82/1260 (6.5%) T-ALL/LBL (7.1% T-ALL and 4.3% T-LBL) and were more frequent within adult T-ALL/LBL as compared to pediatric cases (54/699 adult T-ALL/LBL as compared to 28/561 pediatric cases, $p = 0.05$, supplemental Figure 2). The oncoplot highlighting the main co-mutations observed and mutations within individual *IKZF1*^{Alt} cases are reported in supplemental table S3 and Figure S4.

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 (Table1) and supplemental methods (GRAALL-2003, #NCT00222027; GRAALL-2005, #NCT00327678). Diagnostic peripheral blood or bone marrow samples from 1258 adults and children with T-ALL or LBL-T 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% (26/476), including 16 deletions and 10 mutations. *IKZF1*^{Alt} were observed in 7% of adults and 4.2% of children ($P = 0.2$) with a median age slightly higher in *IKZF1*^{Alt} cases (23.5 years vs. 15.2 years, $P = 0.1$). *IKZF1*^{Alt} was associated with an immature immunophenotype (47% vs. 20%, $P = 0.02$). In line with this, *IKZF1*^{Alt} correlated positively with abnormalities known to be associated with an immature phenotype, including *K/N-RAS* mutations (31% vs. 9%, $P = 0.003$), *EZH2* (5/26, 20% vs 27/450, 6%, $P = 0.02$), *ASXL1* (4/26, 15% vs 15/450, 3%, $P = 0.02$), *ETV6* (4/26, 15% vs 13/450, 3%, $P = 0.01$) and *DNMT3A* (5/26, 20% vs 17/450, 4%, $P = 0.045$) mutations (Figure 1C). Conversely, *IKZF1*^{Alt} were virtually exclusive with *SIL-TAL1+* and *PTEN* altered cases known to be associated with a mature TCR $\alpha\beta$ lineage.

In BCP-ALL, *IKZF1*^{Alt} are enriched in the high-risk subgroup of *Ph*⁺ BCP-ALL and the recently characterized by the presence of a gene expression profile (GEP) 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-ALL²⁰. 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 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 (WBC), central nervous system involvement and prednisone response (Table1). Although *IKZF1*^{Alt} did not impact complete remission rate, patients with *IKZF1*^{Alt} were more likely to have a positive post-induction MRD (10^{-4} threshold, 67% vs 34%; $P = 0.01$). Patients with *IKZF1*^{Alt} had an inferior outcome compared to *IKZF1*^{GL}, with a higher cumulative incidence of relapse (5y-CIR: 50% vs. 28%; specific hazard ratio 2.12, 95%CI[1.17-3.86]) and a shorter overall survival (5y-OS: 37% vs. 73%; hazard ratio: 2.94, 95%CI[1.74-4.96]) (Table1 and Figure 1D-E). This prognostic impact was observed in both pediatric and adult cohorts (supplemental Figures S5A-D). Of note the 10 *IKZF1* mutated and 16 *IKZF1* deleted cases demonstrated comparable clinico-biological features and were associated with worse prognosis when compared to *IKZF1*^{GL} cases (supplemental table S4 and figure S6). In multivariate analysis considering variables associated with OS in univariate analyses as covariates, *IKZF1*^{Alt} remained significantly associated with a shorter OS (Table 1), even after inclusion of post-induction MRD in the model (supplemental table S5). 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 post-induction 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/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 T-ALL.

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Authorship contributions

N.B and 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, N.B provided study materials or patients; M.S, L.L, E.M and V.A performed molecular analyses; M.S, L.L, V.A. collected and assembled data; N.B and M.S performed statistical analysis; M.S, L.L, V.A, N.B analyzed and interpreted data; M.S, N.B, E.M, V.A wrote the manuscript; all authors approved the manuscript.

Disclosure or Conflicts of Interest

The authors declare no competing financial interests.

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. Oncogenetic mutations combined with MRD improve outcome prediction in pediatric T-cell acute lymphoblastic leukemia. *Blood.* 2018;131(3):289–300.
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. 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. 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. Deletion of IKZF1 and Prognosis in Acute Lymphoblastic Leukemia. *New England Journal of Medicine.* 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;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 M-C, Walsh T. Accurate and exact CNV identification from targeted high-throughput sequence data. *BMC Genomics.* 2011;12:184.
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. *J. Clin. Oncol.* 2009;27(6):911–918.
16. Huguet F, Chevret S, Leguay T, et al. 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. MI DB, M van S, Rx DM, et al. A Subtype of Childhood Acute Lymphoblastic Leukaemia With Poor Treatment Outcome: A Genome-Wide Classification Study. *The Lancet. Oncology.* 2009;10(2):.
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. *Leukemia Research.* 2007;31(3):321–327.
23. Kleinmann E, Geimer Le Lay A-S, 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.

Table 1:

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

Abbreviations: T-ALL: T-cell acute lymphoblastic leukemia; WBC, white blood count; CNS, central nervous system; ETP, early thymic precursor; N/F-R/P classifier, *NOTCH1*/*FBXW7*-*RAS*/*PTEN* classifier as previously described; CR, complete remission; MRD, minimal residual disease; HSCT, hematopoietic stem cell transplantation; CIR, cumulative incidence of relapse; OS, overall survival. MRD1 correspond to MRD evaluation after induction and was performed by allele-specific oligonucleotides polymerase chain reaction. T-cell receptor status and oncogenic were performed as described in supplemental methods.

Univariate and Multivariate analyses of Cumulative Incidence of Relapse and Overall Survival:

*continuous variables; **Presence of *RAS*/*PTEN* alteration and/or absence of *NOTCH1*/*FBXW7* mutations. Abbreviations: CIR: cumulative incidence of relapse, OS: overall survival, HR: hazard ratio, SHR: specific hazard ratio, CI: confidence interval.

Figures legends

Figure 1: Figure 1A-E: *IKZF1* mutational and deletion patterns by patient occurrence.

(A). Gene map describing *IKZF1* intragenic mutational patterns by patient occurrence. **(B).** Gene map describing *IKZF1* deletion patterns by patient occurrence. **(C).** Genetic profiles of *IKZF1*^{Alt} T-ALL in the FRALLE and GRAALL 03-05 protocols. Comparison of mutational profiles by 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. Percentage frequencies in each group are indicated. Genes are grouped by functional categories. * p < 0.05. **(D)** Overall survival (OS) and **(E)** Cumulative Incidence of Relapse (CIR) in FRALLE and GRAALL Treated patients.

No (%) of Patients							
Characteristic	IKZF1 ^{Alt}		IKZF1 ^{GL}		Total		p
Total	26	(5.5%)	450	(94.5%)	476	(100%)	
Clinical subsets analyzed							
Male sex	21	(81%)	339	(75%)	360	(75%)	0.6
Median age, years	23.5	[1.1;59.1]	15.2	[1.2;59]	15.4	[1.1; 59.1]	0.1
Median WBC (G/L)	74.1	[2.8;641]	63.4	[0.3;980]	63.8	[0.3; 980]	0.3
CNS involvement	4/26	(15%)	47/448	(11%)	51/474	(11%)	0.5
ETP classification	4/16	(25%)	51/291	(18%)	55/307	(18%)	0.5
T-cell receptor status							
Immature (IM0/δ/γ)	8	(47%)	60	(20%)	68	(22%)	0.02 *
Cortical (IMB, preαβ)	5	(29%)	154	(52%)	159	(51%)	0.08
Mature TCRαβ	3	(18%)	42	(14%)	45	(14%)	0.7
Mature TCRγδ	1	(6%)	39	(13%)	40	(13%)	0.7
Oncogenetics							
TLX1	1	(4%)	53	(14%)	54	(13%)	0.3
TLX3	4	(17%)	68	(17%)	72	(17%)	0.9
SIL-TAL1	2	(8%)	55	(14%)	57	(14%)	0.6
CALM-AF10	1	(4%)	12	(3%)	13	(3%)	0.5
None of above	16	(67%)	202	(52%)	218	(53%)	0.2
HOXA positive	4/21	(19%)	74/315	(23%)	78/336	(24%)	0.8
N/F-R/P classifier							
High Risk classifier	15	(58%)	198	(44%)	213	(45%)	0.2
NOTCH1/FBXW7 ^{mut}	19	(73%)	302	(67%)	321	(67%)	0.7
K/N-RAS ^{mut}	8	(31%)	41	(9%)	49	(10%)	0.003 *
PTEN ^{altered}	2	(8%)	80	(18%)	82	(17%)	0.3
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≥10 ⁻⁴	12/18	(67%)	111/322	(34%)	123/340	(36%)	0.01 *
HSCT	3/24	(13%)	93/416	(22%)	96/440	(22%)	0.3
Outcome							
5-year CIR [95% CI]	50% [32;71]		28% [24;32]		29% [25;33]		0.01 *
5-year OS [95% CI]	37% [19;55]		73% [69;77]		71% [67;75]		<0.001 *
Univariate and multivariate analysis							
CIR	Univariate			Multivariate			
	SHR	95%CI	p	SHR	95%CI	p	
	Age*	1.01 (0.98 ; 1.03)	0.57	-	-	-	-
	Log(WBC)*	1.62 (1.20 ; 2.18)	0.02	1.60 (1.18 ; 2.17)		0.003	
	Prednisone response	0.67 (0.47 ; 0.95)	0.026	0.92 (0.63 ; 1.34)		0.66	
	4-gene classifier**	2.78 (1.94 ; 3.99)	<0.001	2.69 (1.86 ; 3.88)		<0.001	
IKZF1 ^{alt}	2.12 (1.17 ; 3.86)	0.013	2.15 (1.18 ; 3.91)		0.012		
OS	Univariate			Multivariate			
	HR	95%CI	p	HR	95%CI	p	
	Age*	1.03 (1.01 ; 1.05)	0.001	1.04 (1.02 ; 1.06)		<0.001	
	Log(WBC)*	1.99 (1.48 ; 2.67)	<0.001	2.04 (1.49 ; 2.79)		<0.001	
	Prednisone response	0.54 (0.38 ; 0.76)	<0.001	0.79 (0.55 ; 1.15)		0.21	
	4-gene classifier**	2.93 (2.06 ; 4.17)	<0.001	2.88 (2.00 ; 4.16)		<0.001	
IKZF1 ^{alt}	2.94 (1.74 ; 4.96)	<0.001	2.80 (1.61 ; 4.88)		<0.001		

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

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n=82/1260

RNA bp len

200 400 600 800 1,000 1,200 1,400 1,600 1,800

IKZF1 Mutations
42 mutations

Ex1 Ex2 Ex3 Ex4 Ex5 Ex6 Ex7 Ex8

50 100 150 200 250 300 350 400 450 500

6 2

N159T N159S

R164W G190D L192P Q212R G248R G249R V273fs S276fs K273T RS273fs R274fs R279R E304* E304fs G331A Q332* P334fs G337S

F490L R502W G507W S507W ins.LX520

● NONSENSE, n=5
● MISSENSE, n=24
● FRAMESHIFT, n=12
● PROTEIN-TRUNCATING, n=1

■ ZnF domain (DNA binding)
■ ZnF domain (Dimerization)

DEL 1-8 (30/40)

DEL 4-8 (1/40)

DEL 6-8 (1/40)

DEL 2-7 (1/40)

DEL 4-7 (4/40)

DEL 3-5 (1/40)

DEL 1-3 (1/40)

DEL 1 (1/40)

(D) Overall survival (OS) and **(E)** Cumulative Incidence of Relapse (CIR) in FRALLE and GRAALL treated patients.