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► To cite this version:

Mathieu Simonin, Ludovic Lhermitte, Marie-Emilie Dourthe, Etienne Lengline, Carlos Graux, et al.. IKZF1 alterations predict poor prognosis in adult and pediatric T-ALL. *Blood*, 2021, 137 (12), pp.1690-1694. 10.1182/blood.2020007959 . hal-03122574

HAL Id: hal-03122574

<https://hal.science/hal-03122574>

Submitted on 5 Feb 2021

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

Tracking no: BLD-2020-007959R2

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Abstract:

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: 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.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: emails to the corresponding author

Clinical trial registration information (if any): 215 adult patients aged from 16-59 years, were included in the GRAALL03/05 trials which were registered at clinicaltrials.gov (GRAALL-2003, #NCT00222027; GRAALL-2005, #NCT00327678). About 261 pediatric patients aged from 1-19 years, were treated in 10 French pediatric hematology departments, members of the FRALLE study group, according to the FRALLE 2000 T guidelines.

***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.

Acknowledgements

The authors would like to thank all participants in the GRAALL-2003 and GRAALL-2005 study groups, the SFCE and the investigators of the 16 SFCE centers involved in collection and provision of data and patient samples, and V. Lheritier for collection of clinical data. The GRAALL was supported by grants P0200701 and P030425/AOM03081 from the Programme Hospitalier de Recherche Clinique, Ministère de l'Emploi et de la Solidarité, France and the Swiss Federal Government in Switzerland. Samples were collected and processed by the AP-HP "Direction de Recherche Clinique" Tumor Bank at Necker-Enfants Malades. MS was supported by "Soutien pour la formation à la recherche translationnelle en cancérologie dans le cadre du Plan cancer 2009-2013 ». This work was supported by grants to Necker laboratory from the "Association Laurette Fugain", Association pour la Recherche contre le Cancer (Equipe labellisée), Institut National du Cancer PRT-K 18-071 and the Fédération Leucémie espoir and Horizon Hemato.

Authorship contributions

N.B and V.A and M.S conceived the study and oversaw the project; M.S, ME.D, L.L, E.L, C.G, N.G, JM.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.

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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.

Characteristic	No (%) of Patients						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%)	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 *
H SCT	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.

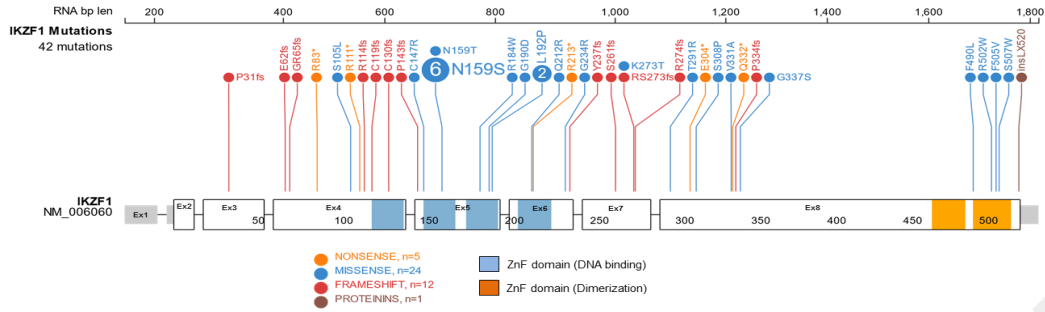
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Figure 1.

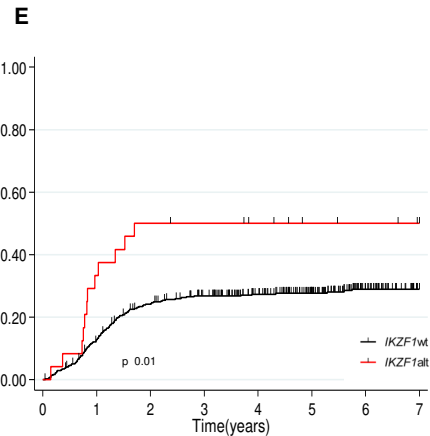
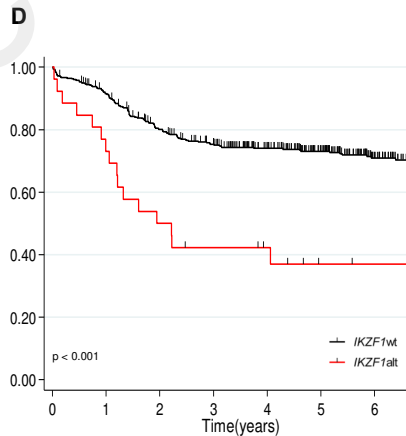
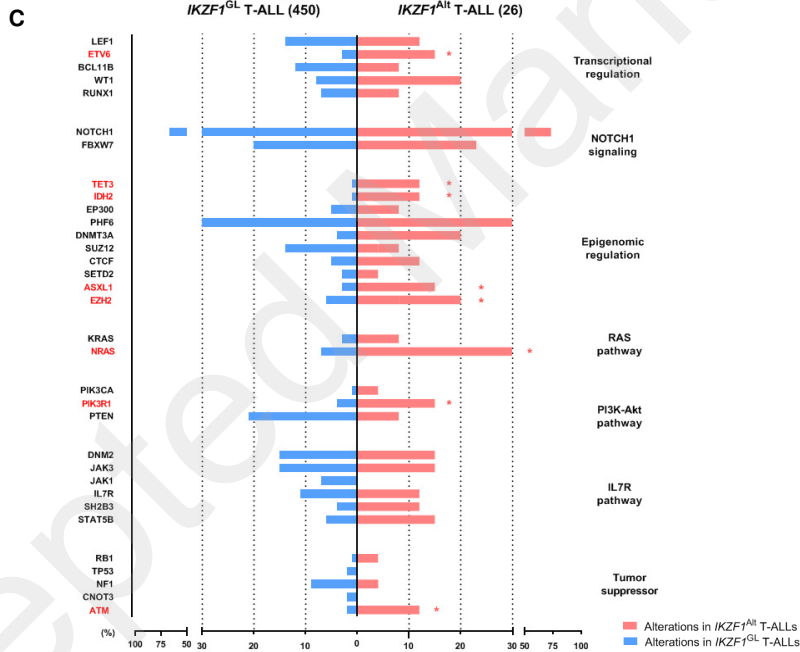
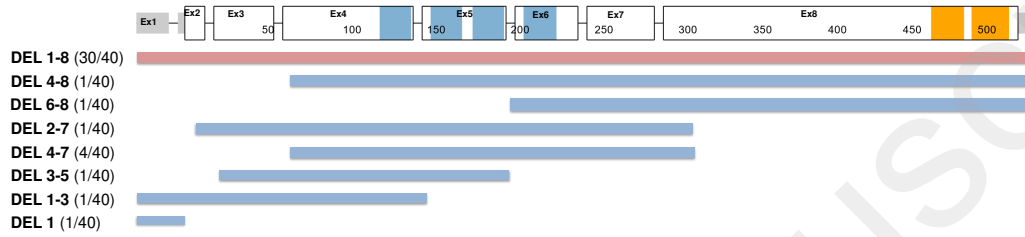
IKZF1 Alterations

n=82/1260

A IKZF1 Mutations (n= 42/82)



B IKZF1 Deletions (n= 40/82)



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