haematologica
Journal of the Ferrata Storti Foundation

B-cell lymphoblastic leukemia/lymphoma with mutated *IKZF1* N159Y: clinical and genetic features of an emerging entity

by Emily N. Alvey, Kai Lee Yap, Pamela Rathbun, Carrie Fitzpatrick, Shunyou Gong and Aida I. Richardson

Received: September 17, 2024.
Accepted: February 17, 2025.

Citation: Emily N. Alvey, Kai Lee Yap, Pamela Rathbun, Carrie Fitzpatrick, Shunyou Gong and Aida I. Richardson. B-cell lymphoblastic leukemia/lymphoma with mutated IKZF1 N159Y: clinical and genetic features of an emerging entity.
Haematologica. 2025 Feb 27. doi: 10.3324/haematol.2024.286626 [Epub ahead of print]

B-cell lymphoblastic leukemia/lymphoma with mutated *IKZF1* N159Y: clinical and genetic features of an emerging entity

Emily N Alvey[1,2], Kai Lee Yap[1,2], Pamela Rathbun[1,2], Carrie Fitzpatrick[3], Shunyou Gong[1,2], and Aida I. Richardson[1,2]

[1]Department of Pathology & Laboratory Medicine, Ann & Robert H. Lurie Children's Hospital of Chicago and [2]Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA; [3]Department of Pathology, University of Chicago, Chicago, Illinois, USA

Authors contributions: E.N.A. analyzed results and wrote the paper; K.L.Y. analyzed and reviewed NGS data; P.R. analyzed and reviewed cytogenetics data; C.F. reviewed, collected, and analyzed clinical and genetic data for patients at University of Chicago; S.G. analyzed clinical and hematopathology data; A.I.R. designed the research and wrote the paper; and all authors edited and approved the final manuscript.

Running head: Characteristics of B-ALL with *IKZF1* N159Y mutation

Corresponding author:
Aida I. Richardson - airichardson@luriechildrens.org/ aida.richardson@northwestern.edu

Disclosures:
Authors have no conflict of interests to disclose.

Data-sharing statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgements: Revised manuscript language was edited by Anne Sally Davis through Eloquenti.

Funding: Revised manuscript language editing was provided by the Stanley Manne Children's Research Institute Research Service Support.

IKZF1 (IKAROS family zinc finger 1) alterations, most commonly partial or full gene deletions, occur in about 15% of pediatric and 40% of adult B-lymphoblastic leukemia/lymphoma (B-ALL) cases and are considered a poor prognostic marker.[1] However, *IKZF1* somatic point mutations are uncommon. B-ALL with mutated *IKZF1* N159Y (p.Asn159Tyr), classified as a new entity per the International Consensus Classification of Myeloid Neoplasms and Acute Leukemia, represents a rare subtype of B-ALL with an estimated incidence of <1% and only a few reported cases.[2-5] This is the first comprehensive study that provides the clinical, immunophenotypic, and genetic features. We found that this rare entity occurs in more than half of pediatric patients, typically presenting with an abnormal karyotype, a gain of chromosome 21, absence of a fusion transcript, and a few additional genetic abnormalities.

We searched our pathology archives, collaborated with nearby colleagues and performed a thorough literature review, identifying in total of 13 patients with an *IKZF1* N159Y mutation. Overall, 2 patients were from our institution, 1 patient was from the University of Chicago, and 10 patients were documented in the literature, with some overlap between two studies and a single case from a different study.[3-5] Clinical and demographic characteristics of these 13 patients were reviewed. The genetic characteristics (conventional cytogenetic, fluorescence in situ hybridization (FISH) and comprehensive next-generation sequencing (NGS) which included analysis of RNA, DNA and copy number variants (CNVs) were analyzed. Classification of variants identified through NGS was performed per the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) criteria. Positive minimal residual disease in all patient samples collected at the end of induction was defined as less than or equal to 0.01% by flow cytometry analysis except for patient #10 where PCR for IgG rearrangement was performed. This study was conducted in compliance with the ethical principles of the Declaration of Helsinki and our institutional review board policy.

Although B-lymphoblastic leukemia/lymphoma (B-ALL) with mutated *IKZF1* N159Y occurred in patients from 2 to 45 years of age with no clear predilection for males or females, more than half of the cases occurred in pediatric patients (61.5%, 8/13) (Figure 1A and Table 1). The pediatric patient group contained standard-risk (SR) and high-risk (HR) patients (4 SR and 3 HR) (Table 1).

Interestingly, patients often presented with white blood cell counts within the normal range (2.8-16.7 K/uL) (67%, 8/12) (Table 1). However, none of the cases identified in the literature provided details regarding clinical presentation and blast immunophenotype. The three patients (patients #11-12 from our institution and patient #13 from University of Chicago) described in the current study presented with anemia (range 6.3 - 7.3 g/dL) and had no evidence of clinical nervous system (CNS) involvement by leukemic cells at the time of diagnosis. Additionally, all three patients exhibited similar blast morphology and immunophenotype. The blasts expressed CD10 (bright), CD19, CD22, CD34, CD38 (dimmer than normal to negative), CD79a, TdT; and were negative for CD20, CD33 and other myeloid and T cell markers. This is the first report highlighting the immunophenotype by flow cytometric analysis for all 3 B-ALL patients with *IKZF1* N159Y. Notably, all patients who had reached the end of induction at the time of review were negative for minimal residual disease (100%, 8/8), and had reached complete remission (100%, 4/4) (Figure 1A and Table 1).

Chromosome analysis revealed that most patients harbored an abnormal karyotype (90%, 9/10) while 83% of patients (10/12) had a gain of chromosome 21 as determined by cytogenetic analysis and/or copy number variations from exome sequencing (Table 2). Notably, additional mutations were uncommon, with pathogenic variants classified as Tier II, variants of potential clinical significance in genes involved in RAS signaling pathway (*KRAS* and *FLT3*) being the most frequent (15%, 2/13) (Table 2). Variants classified as Tier III, variants of uncertain clinical significance (VUSs) observed in this study were either not reported in other databases *(ETV6*, *KMT2D*), previously documented to be germline variants per the ClinVar database (*RUNX1*) or classified as both germline and somatic variants per ClinVar and Cosmic databases (*IL7R* and *NOTCH1*) (Table 2). Computational predictive tools suggest that variants of uncertain significance (*KMT2D*, *RUNX1*, *IL7R* and *NOTCH1*) may not impact protein. Although we cannot know with certainty if these variants are somatic or germline due to the analysis of only neoplastic samples, the allele frequency and available data are more in favor for *RUNX1*, *IL7R* and *NOTCH1* VUS variants being of germline origin. Fusion transcripts were not detected by NGS or reported in the literature (0%, 0/13) (Table 2). Two patients, #6 and #11 showed chromosomal translocations that were detected by chromosome analysis t(6;21)(p23;q11.2) and t(14;21)(q21;q22), respectively. However, it is currently unknown if these translocations generate functional fusion products as they have neither been reported in the literature nor observed with RNA analysis.

This study identified that B-ALL with *IKZF1* N159Y is strongly associated with a gain of chromosome 21. Trisomy of chromosome 21 in the constitutional setting is diagnostic of Down syndrome and is associated with an increased risk of acute lymphoblastic leukemia.[6] Approximately 35% of Down syndrome-associated B-ALL show an *IKZF1* deletion.[7] However, the gain of chromosome 21 in patients with B-ALL with *IKZF1* N159Y is a somatic mutation and thus is not associated with Down syndrome. Furthermore, unlike other *IKZF1* deletions, commonly associated with *BCR-ABL1*-like B-ALL, patients with B-ALL with mutated *IKZF1* N159Y appear to have very few additional genetic abnormalities. Specifically, no fusion transcripts were detected by NGS or reported in the literature.[3-5]

Although this study is limited in the number of patients due to the rarity of this emerging entity being less than 1% of B-ALL, patients with B-ALL with mutated *IKZF1* N159Y, in this small cohort, had a good response to treatment with 100% (8/8) being negative for minimal residual disease at the end of induction and remaining in remission after diagnosis (Table 1). In contrast, many studies have identified *IKZF1* deletions as an adverse prognostic predictor, commonly associated with *BCR-ABL1*-like B-ALL.

IKZF1 is located on chromosome 7p12.2 and consists of 8 exons. Most *IKZF1* deletions occur in exons 4-7 (exons 3-6 in previous nomenclature), resulting in expression of the IK6 isoform, a dominant-negative form of IKAROS, that lacks the DNA-binding domain. *IKZF1* deletions lead to a dominant-negative IKAROS effect, in part by mislocalizing *IKZF1* from the nucleus to the cytoplasm.[8, 9] Exons 4 to 6 are thought to be essential for maintaining *IKZF1* tumor suppressor function. The *IKZF1* N159Y missense variant is located in exon 5, in the DNA-binding domain of *IKZF1*. *IKZF1* missense variants are commonly located at or near residues known to be critical for DNA binding.[10] Previous studies have shown that the *IKZF1* N159Y variant disrupts

IKZF1 function, resulting in distinctive nuclear mislocalization and the induction of aberrant intercellular adhesion characteristic of many *IKZF1* alterations.[9] While *IKZF1* missense variants have a damaging effect on the protein, the missense variants may be less deleterious when compared to partial or whole gene deletions. Also, the deleterious effect of *IKZF1* alterations may be confounded by other co-occurring genomic aberrations. For example, patients with co-occurring *IKZF1* and *ERG* deletion, commonly observed in B-ALL with *DUX4* rearrangement tend to have better clinical outcomes.[11-13] In contrast, *IKZF1* deletions co-occurring with deletions in *CDKN2A, CDKN2B, PAX5*, or the pseudoautosomal region PAR1 in the absence of *ERG* deletion are thought to be adverse prognostic indicators.[14-15] Importantly, B-ALL with mutated *IKZF1* N159Y missense mutation displays a strikingly different gene expression profile compared to other B-ALL cases, including other *IKZF1*-altered cases.[3-4] Notably, B-ALL with mutated *IKZF1* N159Y exhibited upregulation of different genes from those observed to be upregulated in patients with other *IKZF1* alterations and also showed down-regulation of genes involved in B cell receptor signaling and JAK-STAT signaling, such as *FLT3* and *STAT5A*.[3-4]

In conclusion, *IKZF1* N159Y mutation is a rare recurrent abnormality in B-ALL. This unique entity occurs more commonly in pediatric patients and has a strong association with an abnormal karyotype and acquired gain of chromosome 21. Additionally, patients with B-ALL with *IKZF1* N159Y mutation have no detectable fusion transcripts, have very few additional genetic abnormalities, and based on available data, all patients who had reached the end of induction were negative for minimal residual disease.

References

1. Stanulla M, Cavé H, Moorman AV. IKZF1 deletions in pediatric acute lymphoblastic leukemia: still a poor prognostic marker? Blood. 2020;135(4):252-260.
2. Arber DA, Orazi A, Hasserjian RP, et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228.
3. Gu Z, Churchman ML, Roberts KG, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. Nat Genet. 2019;51(2):296-307.
4. Li JF, Dai YT, Lilljebjörn H, et al. Transcriptional landscape of B cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases. Proc Natl Acad Sci USA. 2018;115(50):E11711-E11720.
5. Meyers S, Alberti-Servera L, Gielen O, et al. Monitoring of leukemia clones in B-cell acute lymphoblastic leukemia at diagnosis and during treatment by single-cell DNA amplicon sequencing. Hemasphere. 2022;6(4):e700.
6. Buitenkamp TD, Izraeli S, Zimmermann M, et al. Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. Blood. 2014;123(1):70-77.
7. Buitenkamp TD, Pieters R, Gallimore NE, et al. Outcome in children with Down's syndrome and acute lymphoblastic leukemia: role of IKZF1 deletions and CRLF2 aberrations. Leukemia. 2012;26(10):2204-2211.
8. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5):470-480.
9. Churchman ML, Low J, Qu C, et al. Efficacy of retinoids in IKZF1-mutated BCR-ABL1 acute lymphoblastic leukemia. Cancer Cell. 2015;28(3):343-356.
10. Cobb BS, Morales-Alcelay S, Kleiger G, Brown KE, Fisher AG, Smale ST. Targeting of Ikaros to pericentromeric heterochromatin by direct DNA binding. Genes Dev. 2000;14(17):2146-2160.
11. Clappier E, Auclerc MF, Rapion J, et al. An intragenic ERG deletion is a marker of an oncogenic subtype of B-cell precursor acute lymphoblastic leukemia with a favorable outcome despite frequent IKZF1 deletions. Leukemia. 2014;28(1):70-77.
12. Zaliova M, Zimmermannova O, Dörge P, et al. ERG deletion is associated with CD2 and attenuates the negative impact of IKZF1 deletion in childhood acute lymphoblastic leukemia.[published correction appears in Leukemia. 2015;29:1222]. Leukemia. 2014;28(1):182-185.
13. Lilljebjörn H, Henningsson R, Hyrenius-Wittsten A, et al. Identification of ETV6-RUNX1-like and DUX4-rearranged subtypes in paediatric B-cell precursor acute lymphoblasticleukaemia. Nat Commun. 2016;7:11790.
14. Garcia-Solorio J, Núñez-Enriquez JC, Jiménez-Olivares M, et al. IKZF1plus is a frequent biomarker of adverse prognosis in Mexican pediatric patients with B-acute lymphoblastic leukemia. Front Oncol. 2024; 14:1337954
15. Stanulla M, Dagdan E, Zaliova M, et al. IKZF1plus defines a new minimal residual diseasedependent very-poor prognostic profile in pediatric B-cell precursor acute lymphoblastic leukemia. J Clin Oncol. 2018;36(12):1240-1249.

Table1: Demographic and clinical data

Patient # (Patient ID)/study	Age (years)	Gender	Age Group	Risk stratification for pediatric patients	WBC, $10^3/\mu L$	WBC counts Low/Normal/High	MRD negative <0.01% positive >0.01%	In remission (months)
#1 (SJBALL014062)[3,4]	45	Female	Adult	NA	13.8	High	Negative: 4/4	ND
#2 (SJBALL014288)[3,4]	40	Male	Adult	NA	8.9	Normal		
#3 (SJBALL020602)[3,4]	14	Male	Child	HR	13.5	High		
#4 (SJBALL020623)[3,4]	10	Female	Child	HR	8.1	Normal		
#5 (SJBALL020655)[3,4]	11	Female	Child	HR	6.9	Normal		
#6 (SJBALL 020163)[3]	22	Female	AYA	NA	11.6	High		
#7 (SJBALL 044887)[3]	32	Male	AYA	NA	6.8	Normal		
#8 (SJALL 050830)[3]	8	Male	Child	SR	8.2	Normal		
#9 (C094)[4]	NR	Male	Child	ND	ND	ND	ND	ND
#10 (XG118)[5]	>16	Female	AYA	ND	8.8	Normal	Negative: 1/1	Yes (3 months)
#11	9	Female	Child	SR	2.8	Low	Negative: 3/3	Yes (30 months)
#12	2	Female	Child	SR	16.7	Normal		Yes (30 months)
#13	4	Female	Child	SR	7.7	Normal		Yes (48 months)
Total: 13	Median: 11 Range: 2-45 years	M: 5/13 (38.5%) F: 8/13 (61.5%)	Child: 8/13(61.5%) AYA: 3/13 (23%) Adult: 2/13 (15%)	SR: 4/7 (57%) HR: 3/7 (43%)	Median: 8.5 Range: 2.8-16.7	High: 3/12 (25%) Normal: 8/12 (67%) Low: 1/12 (8%)	Negative: 8/8 (100%) Positive: 0/8 (0%)	4/4 (100%)

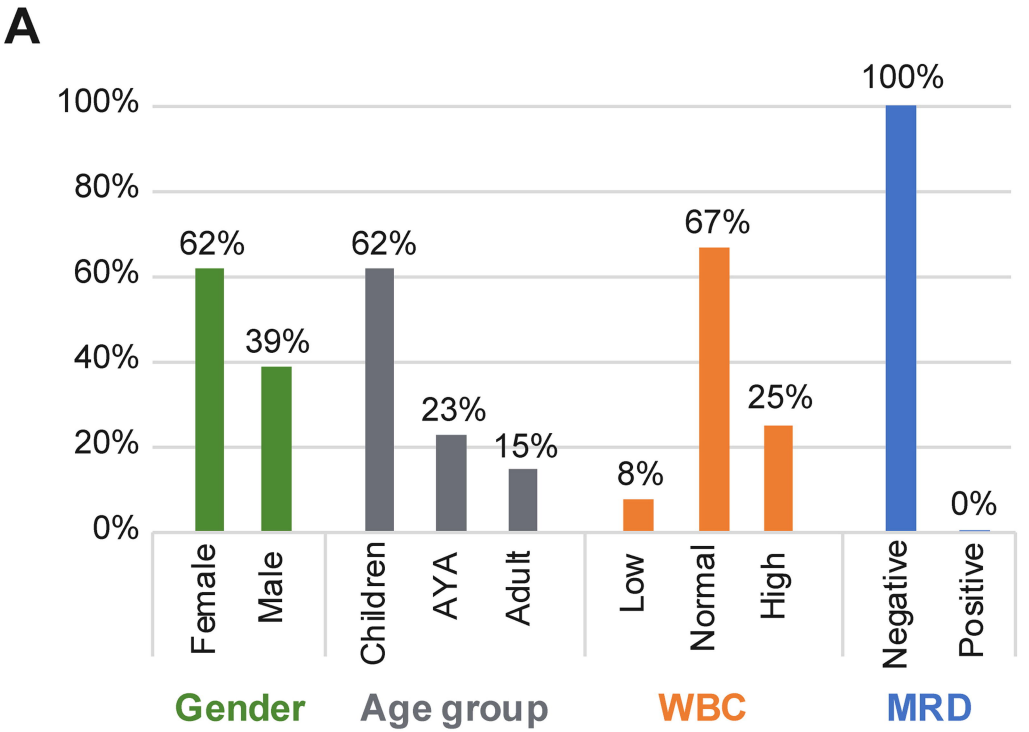
NR, not reported; WBC, white blood cell count; MRD, minimal residual disease; NA, not applicable; ND, not determined; HR, high risk; SR, standard risk; AYA, adolescent and young adults; M, male; F, female; Child, Childhood**.**

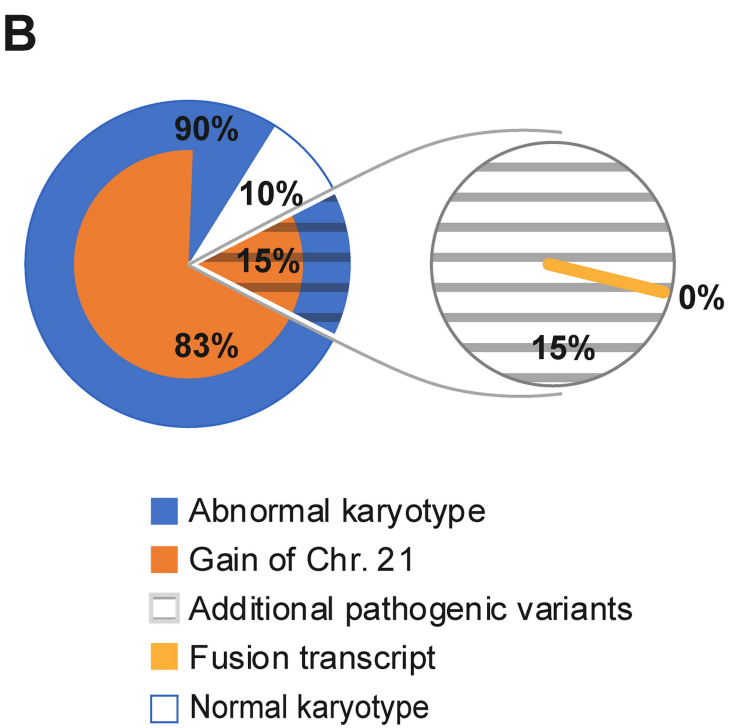
Table 2. Genetic abnormalities (cytogenetic and molecular analysis)

Patient # (Patient ID)/study	Karyotype	Gain of Chr. 21	Fusion	NGS additional genetic aberrations
#1 (SJBALL014062)[3,4]	49,XX,+X,+18,+20[8]/46,XX[11]	No	No	None
#2 (SJBALL014288)[3,4]	47,XY,del(11)(q13q14),add(16)(q22),+21[13]/46,XY[7]	Yes	No	VUS (Tier III): *IL7R* NM_002185.5 p.V272I (c.814G>A) VAF:66%
#3 (SJBALL020602)[3,4]	47,XY,+21[2]/46,XY[18]	Yes	No	None
#4 (SJBALL020623)[3,4]	46,XX[48]	No	No	VUS (Tier III): *ETV6* NM_001987.5 p.D101E (c.303C>A) VAF:12.7%
#5 (SJBALL020655)[3,4]	47,XX,+21[10]/46,XX[1]	Yes	No	Variant of potential clinical significance (Tier II): *KRAS* NM_033360.4 p.L23R (c.68T>G) VAF: 40% VUS (Tier III): *KMT2D* NM_003482.4 p.L4107V (c.12319C>G) VAF:NR
#6 (SJBALL020163)[3]	46~48,XX,add(2)(q33)[2],+6[3],der(6)t(6;21)(p23;q11.2)[10],der(6)t(6;21)x2[3],+21[2][cp15]/46,XX[5]	Yes	No	CNV: *ETV6* (focal 1 copy loss)
#7 (SJBALL044887)[3]	ND	Yes	No	None
#8 (SJALL050830)[3]	ND	Yes	No	None
#9 (C094)[4]	ND	ND	No	None
#10 (XG118)[5]	47,XX,+21[9]/47,sl,add(3)(q13),del(4)(q13q24),add(6)(p11),-12,add(14)(p11),+mar[3]/46,XX[8]	Yes	No	None
#11	46,XX,del(4)(q25q35),add(7)(p11.2),add(10)(q22), der(14)t(14;21)(q21;q22)[14]/46,XX[5]	Yes	No	Variant of potential clinical significance (Tier II): *FLT3* NM_004119.2 p.Y591C (c.1772A>G) VAF:51%
#12	87<4n>,XXX,-X,-2,-4,-11,-12,-15,-17,-18,+21,+21,+21,+21,-22[9]/46,XX[23]	Yes	No	VUS (Tier III): *NOTCH1* NM_017617.5 p.Arg1672His (c.5015G>A) VAF:53.8% *RUNX1* NM_001001890.2 p.Asp96Glu (c.288T>A) VAF:49.8%
#13	47,XX,+21[5]/46,XX[15]	Yes	No	None
Total: 13	9/10 (90%)	10/12 (83%)	0/13 (0%)	2/13 (15%) pathogenic variants

Chr., chromosome; VUS, variant of uncertain significance; ND, not determined; NR, not reported.

Figure 1. Demographic, clinical, and genetic characteristics of B-lymphoblastic leukemia/lymphoma with mutated *IKZF1* N159Y (n=13). (A) Demographic and clinical characteristics. (B) Genetic abnormalities. AYA, adolescent and young adults; WBC, white blood cell count; MRD, minimal residual disease.


A
100%
80%
60%
40%
20%
0%
62%
39%
62%
23%
15%
8%
67%
25%
100%
0%
Female
Male
Children
AYA
Adult
Low
Normal
High
Negative
Positive
Gender
Age group
WBC
MRD


B
90%
10%
15%
83%
0%
15%
Abnormal karyotype
Gain of Chr. 21
Additional pathogenic variants
Fusion transcript
Normal karyotype