The immunophenotypic and genetic characterization of pediatric T-lymphoblastic leukemia with a mature immunophenotype

T-lymphoblastic leukemia (TLBL) is a neoplasm of hematopoietic progenitors committed to the T lineage and accounts for about 15% of childhood leukemias.[1] While extensive immunophenotypic and genomic characterization exists for B-lymphoblastic leukemia, similar reproducibility between these findings in TLBL remains limited. This is largely due to the discordant expression of differentiation markers across various immunophenotypic classification schemes, recapitulating normal complex T-cell differentiation stages.[2] Immunophenotypically, TLBL typically expresses T-cell-associated antigens (CD2, CD5, and CD7) which together typify at least 90% of cases, along with lineage-specific CD3, either cytoplasmic (cCD3) or surface (sCD3), in nearly all cases. The vast majority of TLBL cases show immature T-cell-associated marker expression, such as TdT (in at least 90% of cases), CD1a, CD10, and CD34.[3] In addition to CD34, other aberrant myeloid antigen expressions are seen in early T-cell precursor lymphoblastic leukemia/lymphoma (ETP-ALL). Occasional TLBL cases lack the expression of immature and myeloid markers,[4,5] mimicking mature T-cell neoplasms and posing significant diagnostic challenges. Accurate identification of such cases is critical, particularly in pediatric patients, where prompt diagnosis is essential for timely treatment.

Here, we characterize the features of pediatric TLBL cases lacking the expression of immature markers, with an aim to identify immunophenotypic clues, genetic drivers, and signaling pathway alterations associated with these rare and challenging cases.

This study included 15 cases diagnosed with pediatric TLBL using the World Health Organization (WHO) criteria[6] on peripheral blood (PB) or bone marrow (BM), between January 2012 and July 2024. The study was approved by the Institutional Review Board. Immunophenotype was characterized by flow cytometry, and all cases met the following criteria: (i) positive cCD3 and sCD3; (ii) negative or <10% expression of CD1a, TdT, CD10, and CD34. Genomic analysis including chromosome analysis, fluorescence *in situ* hybridization (FISH), whole genome sequencing (WGS), and RNA-sequencing (transcriptome) were performed in ten cases. Of the remaining five cases, three cases (#3, 4, 6) were analyzed by targeted capture-based next-generation sequencing, with two of these (#4 and #6) also evaluated by optical genome mapping (OGM). The other two cases (#1 and #7) were assessed by conventional cytogenetics and FISH only. T-cell receptor *(TCR*) and immunoglobulin (*IG*) rearrangement analysis were performed using the clonoSEQ® assay.

The patients' characteristics at diagnosis and follow-up are summarized in Table 1. The cohort comprised 12 males and three females (median age, 12 years; range, 1.5-18.0). The BM was diffusely involved in all examined cases (15/15, 100%). PB blast percentages ranged from 0% to 96% (median, 45%); notably, for patients #1, 3, and 13, the original complete blood count (CBC) data were not available, and their CBC data in Table 1 were from a follow-up visit at the time of relapse. Five patients (5/13, 38%) presented with a mediastinal mass. Histologic examination of the BM biopsy showed diffuse or clustered blasts with a high nuclear/cytoplasmic ratio. On BM and PB smears, blasts in all cases showed a similar morphology: small to medium-sized, with round to irregular nuclei, dispersed chromatin, distinct nucleoli, and scant agranular cytoplasm. All 15 patients received TLBL-directed therapy. The median follow-up duration was 15 months (range, 1-123 months). Overall, nine patients had relapsed or refractory disease, while six achieved remission, including three who underwent transplantation (cases #7, #9, and #14 at 5, 7, and 19 months after diagnosis, respectively). At last follow-up, eight patients had died and seven were alive.

At immunophenotypic level, all 15 (100%) cases were positive for cCD3, sCD3, CD5, CD7, and CD45. CD45 was expressed moderately to brightly in 14 (93%) cases, and sCD3 was high (comparable to that of mature reactive T cells) in 11 (73%) cases. Cases were CD8 positive or CD4 and CD8 double-negative (DN) in 12 (80%) and three (20%), respectively. Surface TCR (sTCR) γδ was positive in seven (46%), while sTCR αβ was positive in five (33%) cases. The remaining three cases include one with a co-expression of sTCR αβ and γδ, and two without sTCR expression. Cases with expression of sTCR αβ (TCR αβ +γδ+: 1 case; TCR αβ +γδ-: 5 cases) were more frequent to show expression of CD2, seen in five of six (83%) cases, as compared to only one of the seven sTCR γδ+ αβ - cases (*P*=0.029) (Figure 1). For immunophenotype subtype classification, we performed a hierarchical classification based on the expression status of sTCR, CD4, CD8, and CD2, and classified cases molecularly based on the most recent genomic classification of childhood T-lymphoblastic leukemia by Pölönen *et al*. and Milani *et al*.[7,8] (Figure 1).

Genomic profiling revealed recurrent rearrangements including *STIL::TAL1* in three cases (#1, #2, and #5; #2 with concurrent *LMO2* rearrangement and #5 with concurrent *TRA::MYC*), *KMT2A::AFDN* in two cases (#10 and #11; case #10 with concurrent *SUZ12* truncation), *PICALM::MLLT10* in

two cases (#12 and #13), *TRA/D::MYC* in two cases (#5 and #6; #5 with concurrent *STIL::TAL1*), *STAG2* truncation in one case (#9) and concurrent *ZBTB16::ABL1* and *TCRB::LMO2* rearrangements with ETV6 truncation in one case (#8). In addition, one case (#7) by chromosome analysis showed t(11;14)(p11.2;q32.1), presumably involving *BCL11B* at 14q32. Other common genetic changes included CDKN2A/B (7/13, 53%), *PHF6* and *PTEN* (3/13, 23% each). Copy number analysis revealed CDKN2A/B deletions in seven (53%) cases, with concurrent deletions of both *CDKN2A* and *CDKN2B* identified in six of these cases (85%). *PTEN* alterations were identified in three (23%) cases, *RPL22* variant/deletion, *ETV6, IKZF1, MYB*, and TCF12 variants were identified in one (8%) case each (Figure 1). *NOTCH1* alterations - frequently observed in adult LBL - were detected in only two patients (#9 and #11).

*TCR*γ rearrangement was clonal in all ten tested cases. Four tested sTCRγδ$^+$ cases showed clonal *TCR*γ rearrangement (4/4, 100%, #6, #8, #9 and # 12) with *IGH* rearrangement additionally seen in case #12 and β rearrangement in case #9. Three of four (75%) tested sTCRαβ$^+$ cases showed concurrent *TCR*γ and β rearrangements, while one was clonally γ restricted. The remaining two cases with double-positive (#7) and double-negative (#14) sTCRαβ and γδ expression were clonally γrestricted.

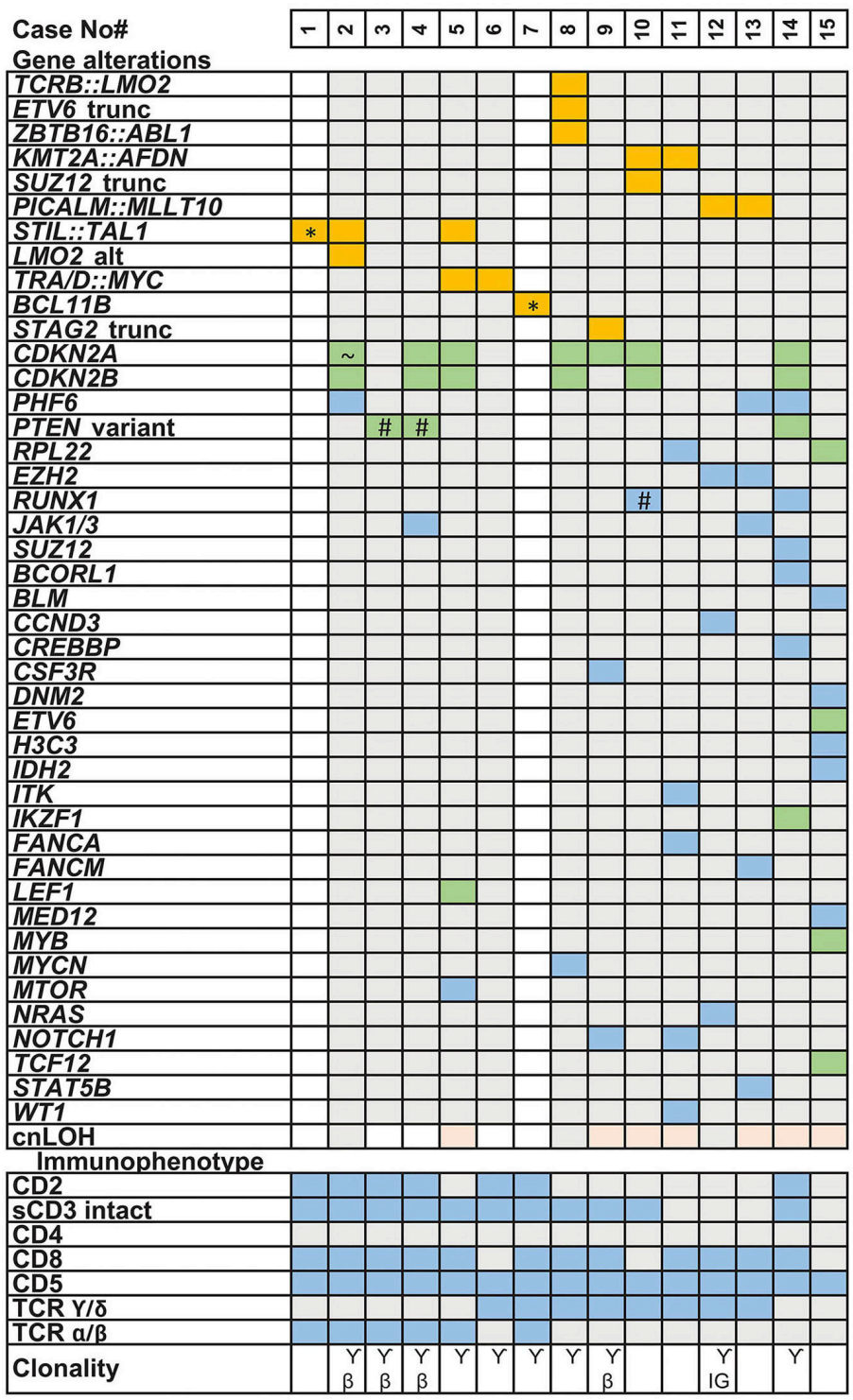
In summary, we characterized 15 cases of TLBL expressing a mature immunophenotype. In contrast to their immature/blastic morphology, these neoplasms lack the expression of immature markers, including CD34, CD1a, CD10, and TdT. Bright CD45 and high sCD3 expressions, similar to mature lymphocytes by flow cytometry, make it difficult to distinguish blasts from mature T cells. The immunophenotypic profile was similar between sTCRαβ$^+$ and sTCRγδ$^+$ cases, except for the expression status of CD2: sTCRγδ$^+$

Table 1. Clinical features of patients with T-lymphoblastic leukemia with a mature immunophenotype.

Case #	Sex	Age, years	F/U, months	Treatment	Response	WBC, x10^9/L	PB blasts, %	Involved sites	LAD	Mediastinal involvement	HSM	Pt status at last F/U
1®	M	14	1	Chemotherapy	Refractory/ relapse	72*	85*	BM, CSF	N/A	N/A	N/A	Dead
2	M	6	19	Chemotherapy	Remission	45.2	22	BM, CSF	No	Yes	Yes	Alive
3®	M	17	7	Chemotherapy	Refractory/ relapse	6*	15*	BM	No	No	No	Dead
4	M	15	22	Chemotherapy	Refractory/ relapse	24.2	74	BM	No	No	Yes	Dead
5®	F	12	15	Chemotherapy	Refractory/ relapse	173	45	BM, CSF	No	No	Yes	Dead
6	M	10	9	Chemotherapy	Refractory/ relapse	125.5	80	BM, CSF	Yes	Yes	Yes	Alive
7	F	15	123	Chemotherapy & SCT	Remission	283.8	96	BM	Yes	No	No	Alive
8	M	2	14	Chemotherapy	Refractory/ relapse	172	85	BM, CSF	Yes	Yes	Yes	Dead
9	M	1.5	72	Chemotherapy & SCT	Remission	1.7	44	BM, CSF	No	No	No	Alive
10	M	7	6	Chemotherapy	Remission	0.4	8	BM	N/A	N/A	N/A	Alive
11	M	11	10	Chemotherapy	Refractory/ relapse	4.9	11	BM, CSF	No	No	No	Dead
12	M	18	1	Chemotherapy	Refractory/ relapse	34.3	43	BM, CSF	Yes	Yes	Yes	Dead
13®	F	5	16	Chemotherapy	Refractory/ relapse	2.9*	0*	BM	Yes	Yes	Yes	Dead
14	M	17	58	Chemotherapy & SCT	Remission	9.9	66	BM, CSF	No	No	No	Alive
15	M	14	46	Chemotherapy	Remission	13	50	BM	No	No	No	Alive

®: relapsed/persistent; BM: bone marrow; CSF: cerebrospinal fluid; F/U: follow-up; F: female; HSM: hepatosplenomegaly; LAD: lymphadenopathy; M: male; N/A: not available (information); PB: peripheral blood; Pt: patient; SCT: stem cell transplant; WBC: white blood cell. *Data from the follow-up visit at the time of relapse.

A



B

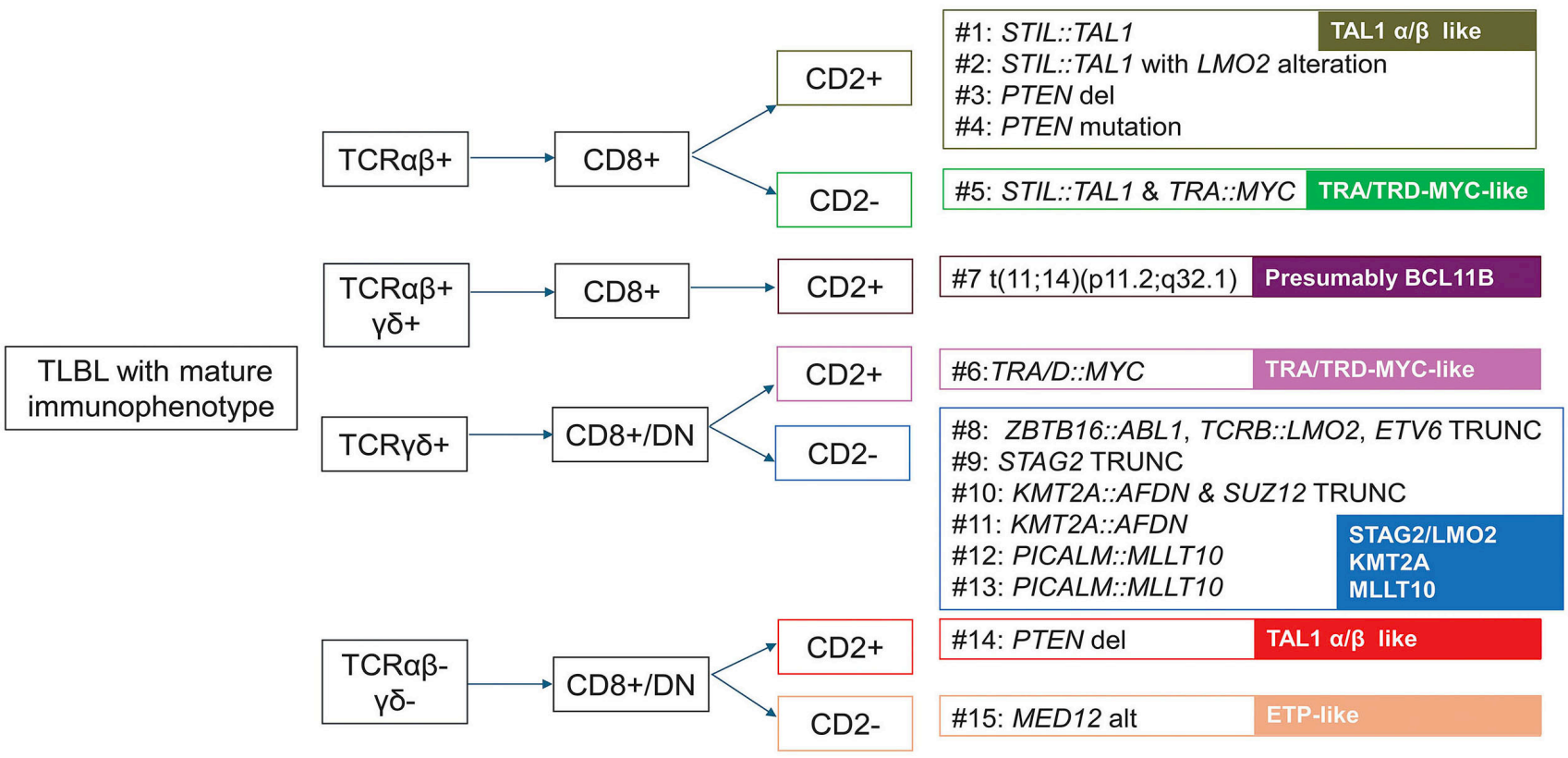


Figure 1. Molecular characterization of 15 cases of T-lymphoblastic leukemia with a mature Immunophenotype. (A) Oncoprint depicting molecular and immunophenotypic features of 15 patients with T-lymphoblastic leukemia with a mature immunophenotype. Every column represents 1 patient; case numbers are provided at the top of the plot. Orange squares indicate the presence of a gene fusion, blue squares indicate the presence of a single nucleotide variants (SNV) or insertion and/or deletion (indel) in the molecular section and positive expression of markers in the immunophenotype section, green squares indicate the presence of copy number variations (CNV), salmon squares indicate the presence of copy-neutral loss of heterozygosity (cnLOH), gray square shows negative for molecular or immunophenotypic markers, and white square indicates not tested. (B) Immunophenotypic findings in correlation with molecular classification of 15 patients with T-lymphoblastic leukemia with a mature immunophenotype. Alt: alteration; Trunc: truncation. *: detected by chromosome analysis and/or fluorescence *in situ* hybridization (FISH); ~: both nonsense variants (SNV) and deletion (CNV) are present; #: different types of variants SNV (#10) or CNV are present (#3, and #4).

mature TLBL more often have $CD2^{-}CD5^{+}$ and express CD7 (bright). Given the negative immature markers and the expression of mature markers, these cases can be potentially misdiagnosed as mature T- or B-cell lymphoma/leukemia. A high index of suspicion for TLBL should be warranted, especially in pediatric patients. Incorporating immunophenotype and associated molecular findings into diagnosis should be considered. Clinical and radiological presentations are also helpful, as the detection of a mediastinal mass raises the possibility of TLBL. Circulating blasts and immature morphology are other supportive features for a diagnosis of TLBL instead of mature T-cell lymphoma/leukemia.

TLBL with an entirely mature immunophenotype has only been rarely reported in children.[4,9] Consequently, the molecular characteristics and clinical features of such cases remain largely unknown. A previous study by Cheng *et al.*[4] described three cases of TLBL with a mature immunophenotype, with the most frequent molecular aberrations being *CDKN2A* deletion and *TRA/D* translocation (each observed in 2 of 3 cases, including 1 case with concurrent *TRA/D::MYC, TCRB::LMO2*, and *CDKN2A* deletion). Two of the three patients in that series either failed to achieve remission or experienced relapse. We explored such cases in more detail in our study, and we found that at molecular level, TLBL cases with a mature immunophenotype are characterized as *NOTCH1*-independent pathways, manifested as frequent loss of the TLBL tumor suppressor genes, such as *CDKN2A/B* (53%), *PTEN* (23%), *RUNX1/BCL11B* (23%), *PHF6* (23%), and *ETV6* (15%). They often display genomic alterations that cause aberrant activation of the *STIL::TAL1/LMO2* oncogenes, *KMT2A* rearrangement, *PICALM::MLLT10* fusion transcript,

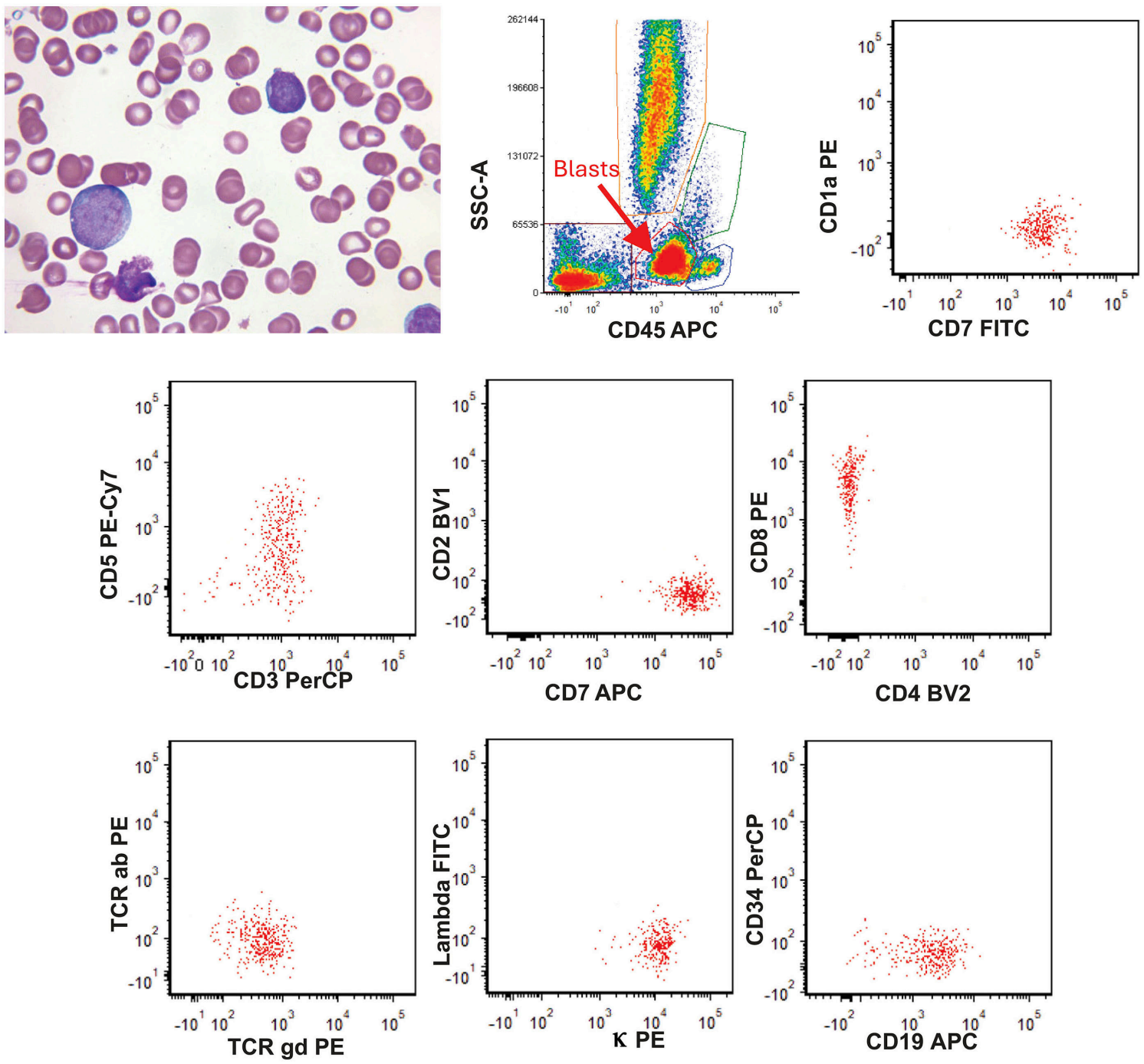


Figure 2. Morphologic and immunophenotypic features of case #13. Aspirate smears show characteristic blasts (Wright-Giemsa), magnification x1,000, oil. Flow cytometry immunophenotyping shows blasts with moderate to bright CD45 expression merging with lymphocytes. Blasts (red color) are positive for surface CD3 (intact, without significant loss), CD5, CD7, CD8, surface TCR γδ, CD19, and surface κ and negative for CD1a, CD2, CD34, surface λ, and TCR αβ.

or *MYC* activation (60%). Previous studies have shown that *TAL1* αβ-like subtype (enriched with *TCRA*-expressing single-positive/mature αβ cells), *LMO2* γδ-like subtype (enriched with δ/effector T cells), and *TRA/D::MYC*-rearranged TLBL mature leukemias cluster close together based on their gene expression signature.[7,10,11]

In all cases described in this study, tumor cells demonstrate T-cell lineage with the expression of both surface and cytoplasmic CD3. In one case (#13), tumor cells showed a mixed phenotype with *PICALM::MLLT10* fusion with blasts expressed both T (sCD3, cCD3, CD5, CD7, and CD8) and B (CD19, CD79a, and κ light chain) markers (Figure 2). Another case with *PICALM::MLLT10* fusion transcript showed both *TCR*γ^+ and *IGH* rearrangements (#12).

Although patients in this study were treated according to different protocols and the cohort size is quite small, the results showed that TLBL with a mature immunophenotype often presented with aggressive disease with poor prognosis, manifested as high white blood cell counts at diagnosis (>100×10^9/L) (4/12, 33%), relapse/refractory (9/15, 60%), and fatality due to leukemia (8/15, 53%). In previous studies, *LMO2* intergenic loss, *TRA/D::MYC*, and *PTEN* deletions were associated with relapse,[4,7,8] consistent with the findings of our study.

Authors

Mahsa Khanlari,[1] Wei Wang,[2] Parastou Tizro[3] and Mohammad K. Eldomery[1]

[1]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN; [2]Department of Hematopathology, MD Anderson Cancer Center, Houston, TX and [3]Department of Hematopathology, City of Hope Comprehensive Cancer Center, Duarte, CA, USA

Correspondence:
M. KHANLARI - mahsa.khanlari@stjude.org

https://doi.org/10.3324/haematol.2025.288233

Received: May 15, 2025.
Accepted: August 1, 2025.
Early view: August 28, 2025.

Disclosures

No conflicts of interest to disclose.

Contributions

All authors wrote and approved the final version of the manuscript. MKE performed molecular data analysis, and MK and WW performed immunophenotypic and data analysis.

Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Raetz EA, Teachey DT. T-cell acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2016;2016(1):580-588.
2. Duffield AS, Mullighan CG, Borowitz MJ. International Consensus Classification of acute lymphoblastic leukemia/lymphoma. Virchows Arch. 2023;482(1):11-26.
3. Noronha EP, Marques LVC, Andrade FG, et al. The profile of immunophenotype and genotype aberrations in subsets of pediatric T-cell acute lymphoblastic leukemia. Front Oncol. 2019;9:316.
4. Cheng J, Wang Y, Gong S, et al. Pediatric T-lymphoblastic leukemia with an entirely mature immunophenotype: a prompt and challenging diagnosis. J Pediatr Hematol Oncol. 2025;47(1):e58-e61.
5. Kimura S, Park CS, Montefiori LE, et al. Biologic and clinical analysis of childhood gamma delta T-ALL identifies LMO2/STAG2 rearrangements as extremely high risk. Cancer Discov. 2024;14(10):1838-1859.
6. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;36(7):1703-1719.
7. Polonen P, Di Giacomo D, Seffernick AE, et al. The genomic basis of childhood T-lineage acute lymphoblastic leukaemia. Nature. 2024;632(8027):1082-1091.
8. Milani G, Matthijssens F, Van Loocke W, et al. Genetic characterization and therapeutic targeting of MYC-rearranged T cell acute lymphoblastic leukaemia. Br J Haematol. 2019;185(1):169-174.
9. Muller J, Walter W, Haferlach C, et al. How T-lymphoblastic leukemia can be classified based on genetics using standard diagnostic techniques enhanced by whole genome sequencing. Leukemia. 2023;37(1):217-221.
10. Homminga I, Pieters R, Langerak AW, et al. Integrated transcript and genome analyses reveal NKX2-1 and MEF2C as potential oncogenes in T cell acute lymphoblastic leukemia. Cancer Cell. 2011;19(4):484-497.
11. La Starza R, Borga C, Barba G, et al. Genetic profile of T-cell acute lymphoblastic leukemias with MYC translocations. Blood. 2014;124(24):3577-3582.