Concurrent peripheral T-cell lymphoma and T-cell lymphoblastic leukemia/lymphoma with identical *STIL::TAL1* fusion events

The World Health Organization and the International Lymphoma Study Group have broadly placed neoplasms with T-cell lineage into lymphomas/leukemias with a precursor T-cell phenotype (e.g., T-lymphoblastic leukemia/lymphoma [T-LBLL]) and those with a mature T-cell phenotype (e.g., peripheral T-cell lymphoma [PTCL]).[1] The accurate classification of lymphoid neoplasms is vital as it determines the subsequent therapy. Historically, in the context of normal T-cell (thymocyte) development, T-LBLL differentiation stages have been identified based on the expression of cluster differentiation (CD) proteins as early, pro-T, pre-T, cortical T, and mature T.[2] Genomic analysis has enabled identifying genetic drivers and signaling pathway alterations associated with different maturational stages of T cell in T-LBLL.[3,4]

Genetic alterations in T-LBLL are mainly composed of master regulators of T-cell fate and differentiation with genes affected in the encoding of transcription factors (e.g., *TAL1/2, LMO1/2, TLX, NKX2-1, BCL11B, HOXA*), along with cooperating abnormalities that influence cell cycle, epigenetic and signal transduction.[5]

Ectopic expression of *TAL1/SCL* (transcription factor located on chromosome 1p33) resulting from *STIL::TAL1* fusion, deletion, or upstream intergenic non-coding mutations is identified in 11-27% of T-LBLL cases.[6,7] *STIL::TAL1*$^{+}$ pediatric T-LBLL cases are heterogeneous in terms of their stages of T-cell development. Although not exclusive, certain types of cooperating abnormalities, such as *PTEN* and *PIK3R* mutations, are more frequently found in *TAL1*-positive cases and correlate with late cortical and mature T-cell immunophenotypes.[3]

PTCL other than anaplastic large-cell lymphoma is rare in pediatric patients and includes PTCL, not otherwise specified (NOS), and mature T/natural killer (NK)-cell neoplasms, characterized by atypical cells with properties of mature T/NK cells.[1,8,9] PTCL-NOS can be further sub-classified into those that highly express one of two transcription factors (GATA-3 or T-bet/TBX21) that regulate normal T-cell differentiation.[10] A heterogeneous genetic landscape of cooperating mutations further characterizes these subtypes in PTCL-not ortherwise specified.[10,11]

Relapses often characterize the natural history of malignant hematopoietic diseases after therapy. The neoplastic clone(s) in relapse are often related to the initial clone(s) but might exhibit additional aberrations, clonal selection, transformation, or phenotypic changes. However, the co-existence of two morphologically and phenotypically distinct but genetically related neoplasms of the T-cell lineage at initial presentation has only been rarely previously reported in T-cell neoplasms. Here, we report on a child presenting with cutaneous T-cell infiltrate diagnosed as PTCL-NOS with concurrent T-LBLL diagnosed in the bone marrow (BM) before the start of therapy. The two tumors shared the same genetic alterations in addition to those uniquely present in each.

The patient was a 10-year-old girl who presented with a single erythematous skin swelling in the mid-back that progressed to an ulcerated mass (6x3 cm) in 3 months. No fever, night sweats, or weight loss were reported. Microscopic evaluation of the lesion showed an atypical infiltrate composed of monotonous cells with a rim of clear cytoplasm, densely clumped chromatin, and small nucleoli. By immunohistochemical stains, the atypical infiltrate was positive for CD2, CD3, CD4 (small subset), CD5, CD8, TIA-1, granzyme B, T-cell receptor (TCR)-β, TBX21, GATA3, and negative for CD1a, CD7, CD10, CD20, Pax-5, CD30, CD34, CD56, CD79a, ALK-1, TCR-δ, TdT, and EBER (*in situ* hybridization) (Figure 1A). The Ki-67 proliferation index was 70%. The overall findings are consistent with PTCL-NOS with CD8^{+} cytotoxic phenotype. With the expression of TBX21,[11] we further subclassified the case as T-bet PTCL subtype. The subsequent positron emission tomography-computed tomography (PET-CT) study for staging showed widespread abnormal uptake in numerous lymph nodes, liver, kidneys, left femur along with a pleural effusion. Complete blood count showed a white blood cell count of 11.23×10^{9}/L, hemoglobin of 95 g/L, and platelet count of 280×10^{9}/L. The patient also had elevated serum lactate dehydrogenase level (1,959 U/L; reference range, 165–310 U/L). BM examination revealed a hypercellular marrow (>95%) with numerous blasts. Blasts were medium in size with a scant amount of basophilic cytoplasm (some vacuolated), irregular nuclear contours, and inconspicuous nucleoli. By immunohistochemical and multicolor flow cytometry immunophenotyping, blasts were positive for CD1a, CD2, cytoplasmic CD3, CD4 (partial), CD7, CD8 (partial), and TdT and negative for surface CD3, CD79a, TCR-β, and TCR-δ (Figure 1B, C). The conventional karyotyping analysis showed 47,XX,+17[8]/46,XX[12]. These findings are diagnostic for T-LBLL. The patient was treated with standard therapy for acute lymphoblastic leukemia. She became minimal residual disease (MRD)-negative by day 15 of therapy and remains in complete remission. The site of excised skin mass healed without complication, and no other skin lesion was detected.

Whole-genome sequencing (WGS), whole-exome sequencing

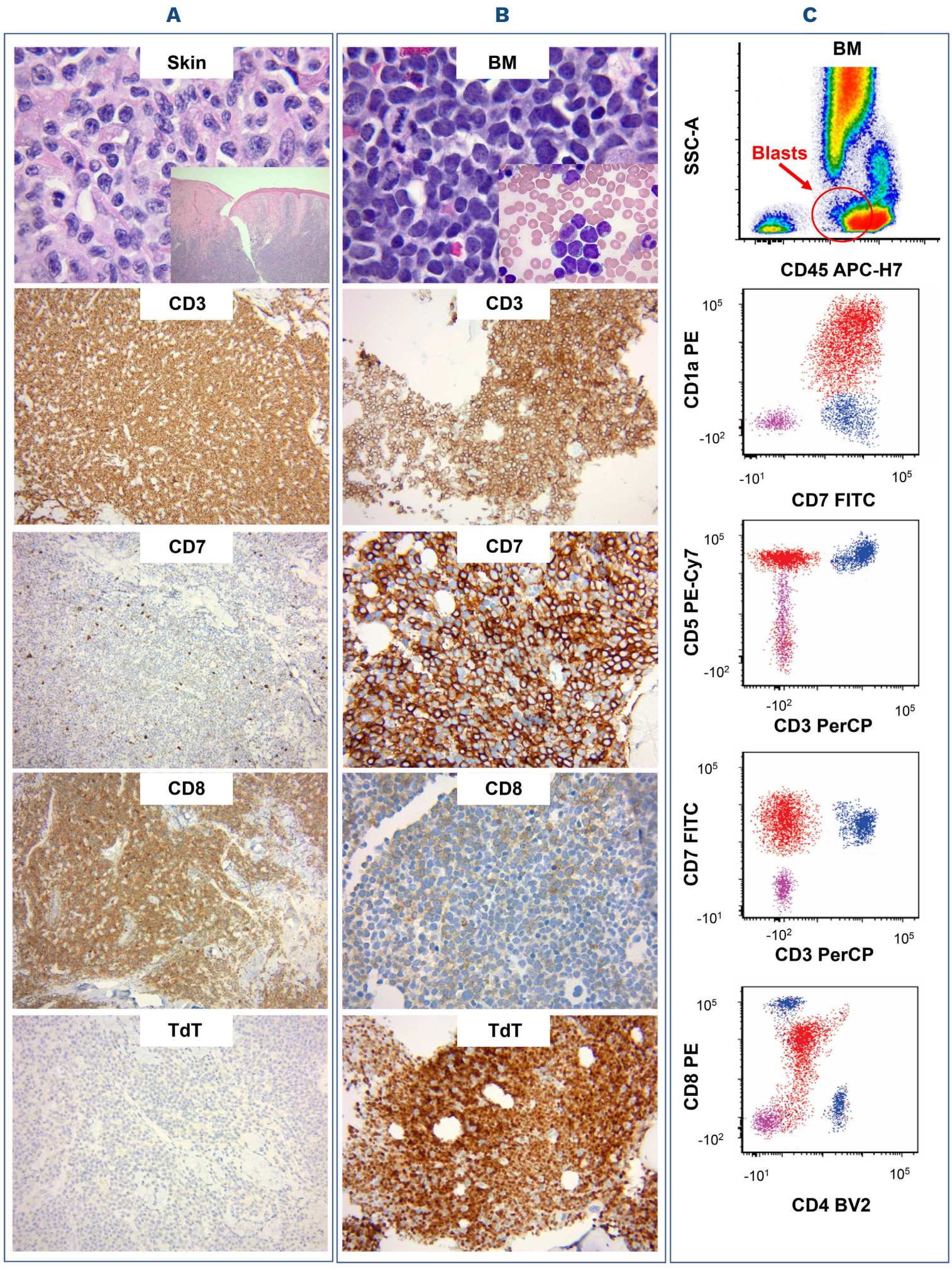

Figure 1. Skin and bone marrow tumor morphology and immunophenotype. (A) Skin biopsy shows infiltration of neoplastic lymphoid cells with a rim of clear cytoplasm, densely clumped chromatin, and small nucleoli (hematoxylin and eosin, x1,000; inset, x40). Immunohistochemical analysis shows the neoplastic lymphoid cells are positive for CD3 (x100), and CD8 (x100) and are negative for CD7 (x100), and TdT (x200). (B) Bone marrow (BM) core biopsy shows a hypercellular marrow diffusely infiltrated by blasts (hematoxylin and eosin stain, x1,000). Aspirate shows medium-sized neoplastic cells with blastoid chromatin and occasional small nucleoli (Wright-Giemsa, inset, x1,000; oil). By immunohistochemical analysis, the blasts are positive for CD3 (x200), CD7 (x400), CD8 (x400), and TdT (x200). (C) BM flow cytometry immunophenotyping analysis demonstrates a distinct population of aberrant T cells (red population) expressing CD45 (moderate expression), CD1a (moderate to bright), CD5 (bright with similar intensity to mature lymphocytes highlighted in blue population), and CD7 (bright). CD4 and CD8 are partially expressed.

(WES), and RNA-sequencing (RNA-seq) were performed on the BM sample along with WES and RNA-seq from formalin-fixed, paraffin-embedded tissue of the skin lesion. The BM and skin tumor specimens shared an identical *STIL::TAL1* fusion (Figure 2A). Both samples had additional 'private' discrete cooperating mutations. The BM sample included mutations in *FBXW7, PIK3CA, KRAS, PTEN, SMARCA4, NOTCH1, BCL11B,* and deletion of *NOTCH1, CDKN2A,* and *CDKN2B*. A

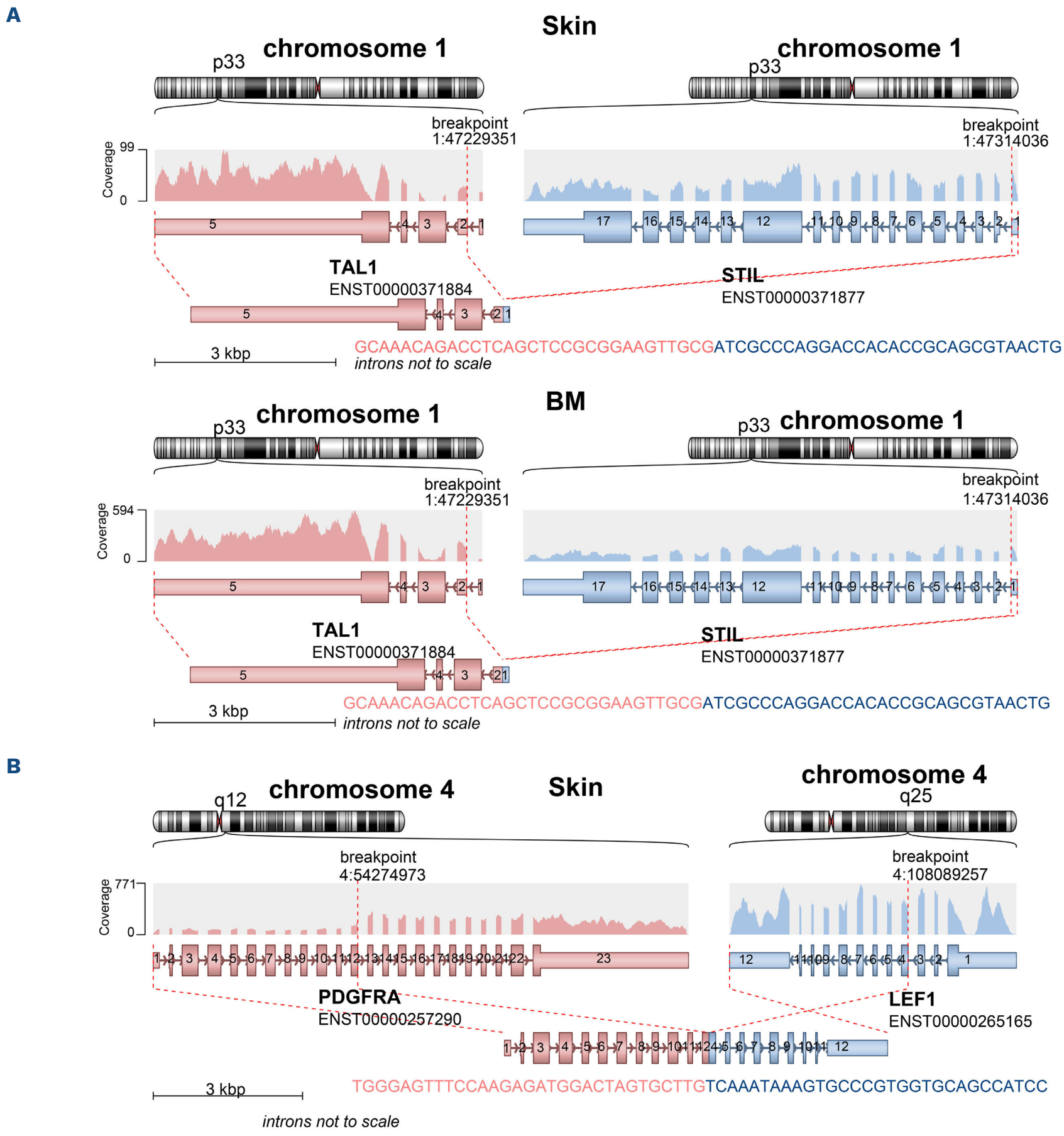

Figure 2. Skin and bone marrow tumor clonal history and novel fusion detected in the skin tumor. (A) The same *STIL::TAL1* fusion transcripts are identified in both skin and bone marrow (BM) samples. These fusion genes are further confirmed using whole-genome sequencing data. For demonstration purposes, only fusion genes detected by RNA-sequencing data are presented in the figure. Fusion breakpoints are labeled according to the hg38 reference. (B) RNA-sequencing analysis of skin and BM samples demonstrate a novel *PDGFRA::LEF1* fusion, identified only in the skin sample, between *PDGFRA* (exon 13) and *LEF1* (exon 3) on chromosome 4. The predicted fusion protein consists of the immunglobulin-like domain of *PDGFRA*, followed by the intact transmembrane domain and tyrosine kinase domain of *PDGFRA*. Because the *LEF1* gene is on the minus strand of chromosome 4, the creation of this fusion appears to have involved an intrachromosomal inversion. RNA read coverage is shown across the genes involved in the fusion.

different *FBXW7* mutation was detected in the skin sample along with a structural variant leading to a *PDGFRA::LEF1* fusion. Targeted resequencing validated these findings and further highlighted the different *FBXW7* mutations in the skin and BM tumors (Figure 3B). By polymerase chain reaction, clonal TCR-γ rearrangement peaks were identified and identical in tumor samples from the skin and BM, confirming their shared origin (Figure 3A).

Despite the significant difference in morphology, immunophenotype, and secondary mutations, the tumors from the BM sample (T-LBLL phenotype) and the skin sample (PTCL, mature T-cell phenotype) had common origins, as confirmed by the shared *STIL::TAL1* fusion and supported by the identical polymerase chain reaction amplification peaks in TCR clonality analysis. Our genomic characterization suggests the clonal relationship likely reflects a common cell of origin with divergent clonal evolution rather than parallel clonal evolution of diverse and unrelated precursors. In the absence of a therapeutic effect, it may be hypothesized that the extrinsic factors, such as cellular environment, immune pressure, cytokine stimulation, etc., differentially impact the tumor cells at distinct sites and promote the evolution of the same clone down different lineage trajectories yielding diverse morphology and immunophenotype. *STIL::TAL1*$^{+}$ T-LBLL have previously been shown to be driven by reiterative mutations of the same driver genes and resultant parallel clonal evolution in which *STIL::TAL1* fusion and *CDKN2A* loss are both early or truncal events while *NOTCH1* and *PIK3/AKT/PTEN* mutations are secondary and subclonal events in the evolutionary pathway.[12] The heterogeneous nature of *STIL::TAL1*$^{+}$ T-LBLL cases with respect to their immunophenotype has also been previously demonstrated in which *TAL1*$^{+}$ cases clustered into two subgroups: double positive (DP, CD4^{+} CD8^{+}) T-LBLL cases with a higher surface CD3 expression and a second smaller group with less surface CD3 expression.[13] Subsequent evaluation of

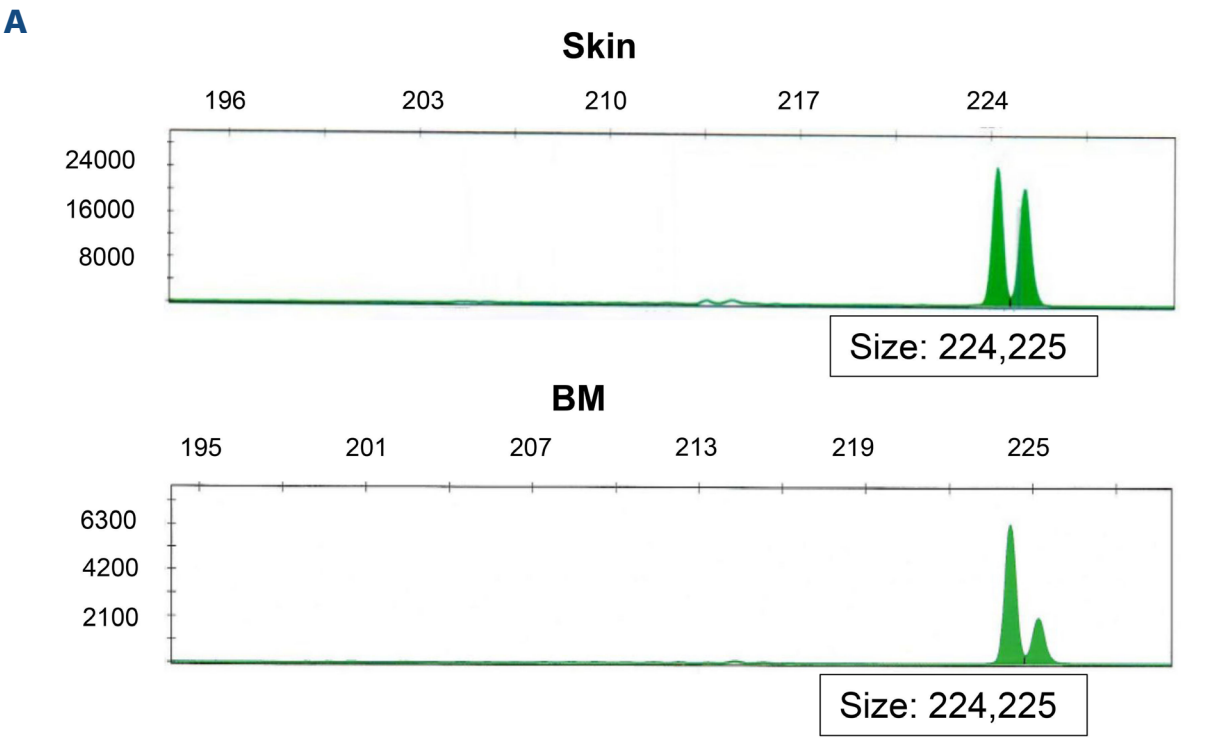

Figure 3. Skin and bone marrow tumor clonal history by T-cell receptor γ gene rearrangement studies by polymerase chain reaction and proposed model of the evolutionary pathway. (A) T-cell receptor γ gene rearrangement studies by polymerase chain reaction, performed on the skin and bone marrow (BM) tumor samples, reveal a monoclonal rearrangement with identical polymerase chain reaction amplification peaks. (B) Evolutionary pathway from a common progenitor with a common clone containing *STIL:: TAL1* event is demonstrated. The molecular alterations in different samples (skin and BM) are highlighted according to the different cellular machineries. The allele frequency of altered genes is represented in parenthesis. The common molecular alteration in skin and BM, *STIL::TAL1* del(1)(p33), appears in bold text.

these clusters found the first cluster of T-LBLL cases containing mature T-LBLL (DP, CD4$^+$ CD8$^+$) was enriched with deletions in *LEF1, CASP8AP2, and CDKN2A/B* in addition to *STIL::TAL1* gene fusion.[14]

Extranodal presentation of this case further adds to the challenges in proper classification and diagnosis. While the incidence rate of all cutaneous T-cell lymphomas in the pediatric age group is low, most cases are within the category of mature T-cell neoplasms. In contrast, skin is infrequently involved by precursor lymphoblastic lymphomas and rarely in T-LBLL.[15] There is also limited data on the molecular analysis of T-LBLL cases presenting with the skin lesion.[16]

In this case, a novel *PDGFRA::LEF1* fusion was detected by sequencing analysis in skin sample with a breakpoint within exon 13 of the *PDGFRA* gene and exon 3 of the *LEF1* gene resulting from an intrachromosomal inversion on chromosome 4q, which caused an out-of-frame fusion (Figure 2B). While the significance of this structural variant cannot be determined, there was insufficient evidence to support an activating *PDGFRA* rearrangement. This alteration spanning over multiple *LEF1* exons can potentially disrupt the *LEF1* gene, leading to its loss of function. It is hypothesized that *LEF1* can play multiple roles in T-cell leukemia as a cooperative tumor suppressor or oncogene.[17] As a tumor suppressor (and possible transcriptional repressor of *MYC*), it is inactivated in approximately 11% of pediatric T-LBLL. Interestingly, *LEF1*-inactivated T-LBLL has been characterized by the lack of CD34 expression and arrest at a transition from CD8$^+$ immature single positive stage to double positive stage.[18] This finding may potentially explain the mature immunophenotype of T-cell infiltrate in the skin sample compared to the BM tumor.[18]

In summary, this case expands the complexity of cases with *STIL::TAL1* fusion to T-cell neoplasms with mature phenotypes. A potential collaborating effect exists between the *STIL::TAL1* fusion and gene abnormalities that shape the T-cell neoplasm's morphologic and immunophenotypic features. Classification and diagnosis of T-cell neoplasms based on morphology and immunophenotypic features alone may be inadequate to fully characterize the ill-defined boundary between mature T-LBLL and PTCL. Primary cutaneous T-cell lymphoma/leukemia is rare in pediatric patients, and due to the rarity of these cases, *TAL-1/2* is not routinely tested. However, our study draws attention to this rare event, and comprehensive cytogenetic and molecular studies would be indicated in extranodal and cutaneous T-cell infiltrates in pediatric patients.

Authors

Mahsa Khanlari,[1] Wei Wang,[2] Yen-Chun Liu,[1] Lu Wang,[1] Jeffrey E. Rubnitz,[3] Stephanie Dixon,[3] Brent A. Orr,[1] Obianuju M. Anelo,[4] Zhongshan Cheng,[5] Vidya Balagopal[1] and Jeffery M. Klco[1]

[1]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN; [2]Department of Hematopathology, MD Anderson Cancer Center, Houston, TX; [3]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN; [4]Department of Pathology, University of Tennessee Health Science Center, TN and [5]Center for Applied Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN, USA

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

https://doi.org/10.3324/haematol.2023.283585

Received: May 23, 2023.
Accepted: September 18, 2023.
Early view: September 28, 2023.

Disclosures

No conflicts of interest to disclose.

Contributions

All authors wrote and approved the final version of the manuscript. ZC and VB performed molecular research 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. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. Leukemia. 2022;36(7):1720-1748.
2. Shiraz P, Jehangir W, Agrawal V. T-cell acute lymphoblastic leukemia-current concepts in molecular biology and management. Biomedicines. 2021;9(11):1621.
3. Liu Y, Easton J, Shao Y, Maciaszek J, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet. 2017;49(8):1211-1218.
4. Khanam T, Sandmann S, Seggewiss J, et al. Integrative genomic analysis of pediatric T-cell lymphoblastic lymphoma reveals candidates of clinical significance. Blood. 2021;137(17):2347-2359.
5. Tan TK, Zhang C, Sanda T. Oncogenic transcriptional program driven by TAL1 in T-cell acute lymphoblastic leukemia. Int J Hematol. 2019;109(1):5-17.
6. Mansur MB, Emerenciano M, Brewer L, et al. SIL-TAL1 fusion gene negative impact in T-cell acute lymphoblastic leukemia

outcome. Leuk Lymphoma. 2009;50(8):1318-1325.
7. D'Angio M, Valsecchi MG, Testi AM, et al. Clinical features and outcome of SIL/TAL1-positive T-cell acute lymphoblastic leukemia in children and adolescents: a 10-year experience of the AIEOP group. Haematologica. 2015;100(1):e10-e13.
8. Hutchison RE, Laver JH, Chang M, et al. Non-anaplastic peripheral T-cell lymphoma in childhood and adolescence: a Children's Oncology Group study. Pediatr Blood Cancer. 2008;51(1):29-33.
9. Ravichandran N, Uppuluri R, Vellaichamy Swaminathan V, et al. Management of peripheral T-cell lymphoma in children and adolescents including STAT 3 mutation hyper-IgE syndrome: one size does not fit all. J Pediatr Hematol Oncol. 2022;44(4):e849-e854.
10. Heavican TB, Bouska A, Yu J, et al. Genetic drivers of oncogenic pathways in molecular subgroups of peripheral T-cell lymphoma. Blood. 2019;133(15):1664-1676.
11. Amador C, Greiner TC, Heavican TB, et al. Reproducing the molecular subclassification of peripheral T-cell lymphoma-NOS by immunohistochemistry. Blood. 2019;134(24):2159-2170.
12. Furness CL, Mansur MB, Weston VJ, et al. The subclonal complexity of STIL-TAL1+ T-cell acute lymphoblastic leukaemia. Leukemia. 2018;32(9):1984-1993.
13. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell. 2002;1(1):75-87.
14. 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.
15. Kempf W, Kazakov DV, Belousova IE, Mitteldorf C, Kerl K. Paediatric cutaneous lymphomas: a review and comparison with adult counterparts. J Eur Acad Dermatol Venereol. 2015;29(9):1696-1709.
16. Vezzoli P, Novara F, Fanoni D, et al. Three cases of primary cutaneous lymphoblastic lymphoma: microarray-based comparative genomic hybridization and gene expression profiling studies with review of literature. Leuk Lymphoma. 2012;53(10):1978-1987.
17. Carr T, McGregor S, Dias S, et al. Oncogenic and tumor suppressor functions for lymphoid enhancer factor 1 in E2a(-/-) T acute lymphoblastic leukemia. Front Immunol. 2022;13:845488.
18. Gutierrez A, Sanda T, Ma W, et al. Inactivation of LEF1 in T-cell acute lymphoblastic leukemia. Blood. 2010;115(14):2845-2851.