CASE REPORT OPEN ACCESS

Concurrent Bone Marrow Acute Undifferentiated Leukemia and Mediastinal T-Lymphoblastic Lymphoma With Identical *SET::NUP214* Fusion and *PHF6* and *EZH2* Mutations

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ABSTRACT

Acute undifferentiated leukemia (AUL) is a rare hematologic malignancy lacking lineage-specific markers. Concurrent, clonally related AUL and T-lymphoblastic lymphoma (T-LBL) has not been reported previously. Here we describe a patient who was diagnosed with AUL in the bone marrow and T-LBL in the mediastinum after a thorough immunophenotyping by flow cytometry and immunohistochemistry. Despite their immunophenotypic differences, the AUL and T-LBL showed identical genetic alterations: *SET::NUP214* fusion, *PHF6*, and *EZH2* mutations. The patient achieved and remained in complete remission after chemotherapy and stem cell transplantation. This case underscores the value of comprehensive immunophenotyping and genetic analysis in rare hematologic malignancies.

Acute undifferentiated leukemia (AUL) is a rare hematologic malignancy characterized by the absence of lineage-specific markers. According to 5th edition of the World Health Organization Classification (WHO-HAEM5) and the 2022 International Consensus Classification (ICC) of hematolymphoid tumors, AUL is defined as blasts expressing no definitive myeloid or lymphoid markers, and fewer than two myeloid-associated markers (CD117 or bright CD13/CD33) [1, 2]. Despite its rarity, mutations in *PHF6*, *SRSF2*, *RUNX1*, and *ASXL1* and gene fusions such as *SET::NUP214* have been reported in AUL [2, 3].

The WHO-HAEM5 and 2022 ICC classifications recognize the heterogeneity of T-lymphoblastic leukemia/lymphoma (T-ALL/LBL). Whereas the WHO-HAEM5 does not provide subclassification for T- ALL/LBL, the ICC introduces eight provisional categories based on aberrant expression of transcription factors, including *HOXA* dysregulated, *SPI1*, *TLX1*, *TLX3*, *NKX2*, *TAL1/TAL2*, *LMO1/LMO2*, and *LYL1*/LMO2 [4]. T-ALL/LBL with *SET::NUP214* fusion results in HOXA activation [5] and is categorized in the *HOXA* subgroup [4].

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Clonally related neoplasms sharing the same gene fusion in a single patient have been described in the literature. Examples include T-ALL/LBL and peripheral T-cell lymphoma (PTCL) with identical *STIL::TAL1* fusions [6], and acute myeloid leukemia (AML) and PTCL with identical *SET::NUP214* fusions [7]. Although *SET::NUP214* fusion can be seen in AUL as well as T-ALL/LBL, respectively, concurrent AUL and T-LBL/ALL sharing identical *SET::NUP214* fusion has not been reported in the literature. Here we report an unusual case of AUL involving the bone marrow with concurrent T-LBL involving the mediastinum. Despite significant differences in immunophenotype, both neoplasms shared the same genetic/molecular alterations, including *SET::NUP214* fusion and mutations in *PHF6* and *EZH2*, supporting their clonal relationship.

A 31-year-old previously healthy man presented with fatigue and dyspnea for 2 months. Positron emission tomography/computed tomography showed a 7.3 cm anterior mediastinal mass. Initial laboratory tests revealed pancytopenia (hemoglobin 7.4 g/dL, platelets 93 K/μL, white blood cells 2.2 K/μL) with no circulating blasts. A bone marrow aspiration and biopsy revealed markedly increased blasts with an interstitial pattern (Figure 1A1). The blasts were intermediate size to large with irregular nuclei and scant cytoplasm; no Auer rods were identified (Figure 1A1 inset). The blasts were negative for myeloperoxidase (MPO) by cytochemistry. Flow cytometric immunophenotypic analysis showed that the blasts were positive for CD34, CD117, CD38 (bright), HLA-DR (partial), CD7 (bright), CD33 (partial), CD10 (partial), and CD123 (partial) and were negative for TdT, CD1a, CD2, CD3 (cytoplasmic and surface), CD4, CD5, CD8, CD56, myeloid markers (MPO, CD13, CD14, CD15, CD64, CD133), and B-cell markers (CD19, CD20, CD22, cytoplasmic CD79a, cytoplasmic IgM) (Figure 2A1–E1). Immunohistochemical analysis of the bone marrow biopsy specimen showed that the blasts were positive for CD117, CD34, and CD7 (Figure 1B1–D1) and were negative for CD3 and BCL11b (Figure 1E1 and F1). Chromosomal analysis of the bone marrow aspirate showed a complex karyotype in near-teraploidy cell populations: 93∼98, XXYY,+X,+1, del(1)(q21q42)x3,-5, 5,+del(9)(q12q32), del(9)(q12q32)x2, del(9)(q34q34)x2,+10,-12,+2∼3mar[cp4]/46, XY[16]. A diagnosis of AUL was rendered in the bone marrow.

Simultaneously, a biopsy of the mediastinal mass revealed diffuse sheets of intermediate-size neoplastic cells with irregular nuclear contours and distinct nucleoli (Figure 1A2). Flow cytometry immunophenotypic analysis showed an aberrant population that was positive for CD1a (dim), cytoplasmic CD3, CD5, CD7, CD13, and CD33 and was negative for CD2, surface CD3, CD4, CD8, CD10, CD19, CD34, CD38, HLA-DR, and TdT (Figure 2A2–E2). Immunohistochemical analysis showed that the neoplastic cells were positive for CD3, CD7, CD117, and BCL11b (Figure 1B2, D2–F2) and were negative for PAX5, CD79a, MPO, CD11c, and CD34 (Figure 1C2). A diagnosis of T-LBL was established for the mediastinal mass.

RNA sequencing and DNA next-generation sequencing were performed on the bone marrow and mediastinal mass specimens. Both the bone marrow and mediastinal specimens showed the same *SET::NUP214* fusion gene [the 5' of *SET* (exons 1–7) fused to the 3' of *NUP214* (exons 8–36)], along with frameshift mutations of *PHF6*(NM_032458.3):c.381_386delinsGCAA;(NP_115834.1):p.(Tyr127*) and *EZH2* (NM_004456.5):c.2187dup;(NP_004447.2):p.(Asp730*). The presence of the *SET::NUP214* fusion gene correlates with the del(9)(q34q34) detected by karyotyping of the bone marrow aspirate.

The patient was treated with cyclophosphamide, vincristine, doxorubicin, and dexamethasone for five cycles and achieved complete remission. He subsequently underwent allogeneic stem cell transplantation in the 6th month after initial diagnosis. He remained in complete remission for at least 10 months after stem cell transplantation.

This case illustrates concurrent AUL in the bone marrow and T-LBL in the mediastinum, a previously unreported combination at presentation. Immunophenotypically, these two neoplasms have some overlapping features: both neoplasms were positive for CD7, CD33, and CD117 and were negative for CD2, CD4, and CD8. However, AUL expressed more markers of immaturity including CD34, CD38 and HLA-DR and did not express cytoplasmic CD3. By contrast, the T-LBL showed evidence of T-cell differentiation positive for CD1a, cytoplasmic CD3, CD5, and BCL11B and aberrant expression of myeloid markers (CD13). Despite these differences, both neoplasms shared identical genetic/molecular alterations supporting a clonal relationship.

NUP214 (nucleoporin 214), located at chromosome 9q34, is a nucleoporin involved in nucleocytoplasmic transport, including mRNA export, and is essential for human chromosome region maintenance 1-mediated nuclear protein export. *NUP214* plays a critical role in development and has been increasingly implicated in leukemogenesis, including fusion events of *DEK::NUP214* in AML [8], *NUP214::ABL1* in T-ALL [9] and *SET::NUP214* in a variety of types of acute leukemia [3, 6, 8]. *SET* (SET nuclear protooncogene), one of the *NUP214* fusion partner genes, functions as an inhibitor of histone acetylation, particularly targeting histone H4, by preventing histone acetyltransferases from acetylating nucleosomes. *SET::NUP214* is a rare fusion typically associated with T-ALL/LBL, but also has been observed in AUL [5], AML, and mixed phenotype acute leukemia [3, 8]. *SET::NUP214* fusion results in upregulation of *HOXA* and *HOXB* and a blockade in cellular differentiation [5]. However, *SET::NUP214* fusion is insufficient to induce leukemogenesis on its own and requires additional molecular and chromosomal events for full transformation [10]. In the present case, the co-occurrence of mutations in *PHF6* and *EZH2* and the complex karyotype in AUL likely contributed to the development of leukemia. Both *PHF6* and *EZH2* are involved in epigenetic regulation. *PHF6* mutations are commonly found in T-ALL/LBL cases with the *SET::NUP214* fusion [11] and have been identified in 33% of AUL cases. Similarly, mutations involving components of the polycomb repressive complex 2 (PRC2), including *EZH2*, have been implicated in up to 25% of T-ALL/LBL cases [12]. However, mutation of *EZH2* has not been reported in AUL [2].

In normal hematopoiesis, early progenitors from the bone marrow migrate to the thymus to undergo T-cell differentiation, suggesting the critical impact of microenvironment on cell differentiation. Similarly, the tumor microenvironment is composed of fibroblasts, blood vessels and white blood cells, particularly T-cells and macrophages and plays an important

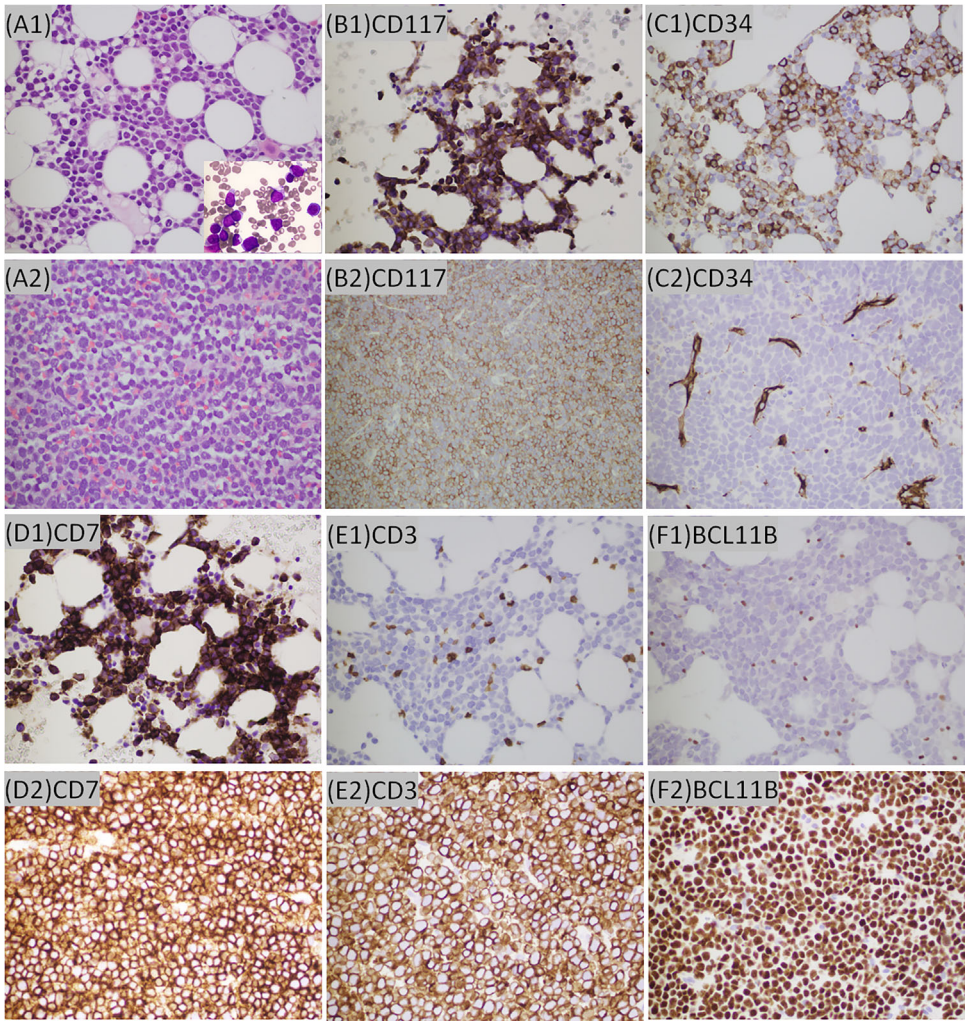

FIGURE 1 | Comparison of morphologic and immunohistochemical findings in bone marrow and mediastinal mass. (A1) The bone marrow biopsy specimen shows markedly increased blasts distributed in an interstitial pattern. On aspirate smears, the blasts are intermediate-size to large with irregular nuclei and scant cytoplasm (inset). (**A2**) The mediastinal mass biopsy specimen shows a diffuse proliferation of intermediate-size neoplastic cells with irregular nuclear contours and distinct nucleoli. (**B1–F1**) The blasts in bone marrow are positive for CD7, CD34, and CD117 and are negative for CD3 and BCL11B. (**B2–F2**) The neoplastic cells in mediastinal mass are positive for CD3, CD7, CD117, and BCL11B and are negative for CD34. (A) Hematoxylin-eosin stain, original magnification ×400. A1 insert, Wright–Giemsa stain, original magnification ×500. (B–F) Immunohistochemistry, original magnification ×400.

role in leukemia development and progression [13]. It seems possible that the undifferentiated (stem cell-like) blasts of the AUL in this patient may migrated from the bone marrow to his thymus where the environmental factors induced the blasts to differentiate towards T-cell lineage, resulting in T-LBL. A similar hypothesis has been proposed by others who reported a patient with T-ALL/LBL in the bone marrow with coexistent, clonally related PTCL involving the skin [7].

The presence of *SET::NUP214* fusion is often associated with a poor prognosis, particularly in patients with T-ALL/LBL, where the fusion has been linked to chemotherapy resistance and a higher likelihood of relapse [14]. The co-occurrence of *PHF6* mutation further complicates the clinical course, as these mutations also have been associated with poor outcomes in various hematologic malignancies [15]. Therefore, patients with *SET::NUP214* fusion and *PHF6* mutation may need more aggressive treatment. The patient we report here has achieved and remained in complete remission after hyper-CVAD chemotherapy and stem cell transplantation.

This case highlights a very rare presentation of concurrent AUL and T-LBL with each neoplasm sharing the same *SET::NUP214* fusion and *PHF6* and *EZH2* mutations. We hypothesize that bone marrow blasts of AUL migrated to the thymus where the microenvironment promoted T-cell differentiation and T-LBL. Comprehensive immunophenotyping was required for establishing the correct diagnoses in this patient. Identification of genetic/molecular alterations was crucial for understanding the relationship between these two neoplasms and for guiding therapeutic decisions, as these alterations are associated with poor prognosis.

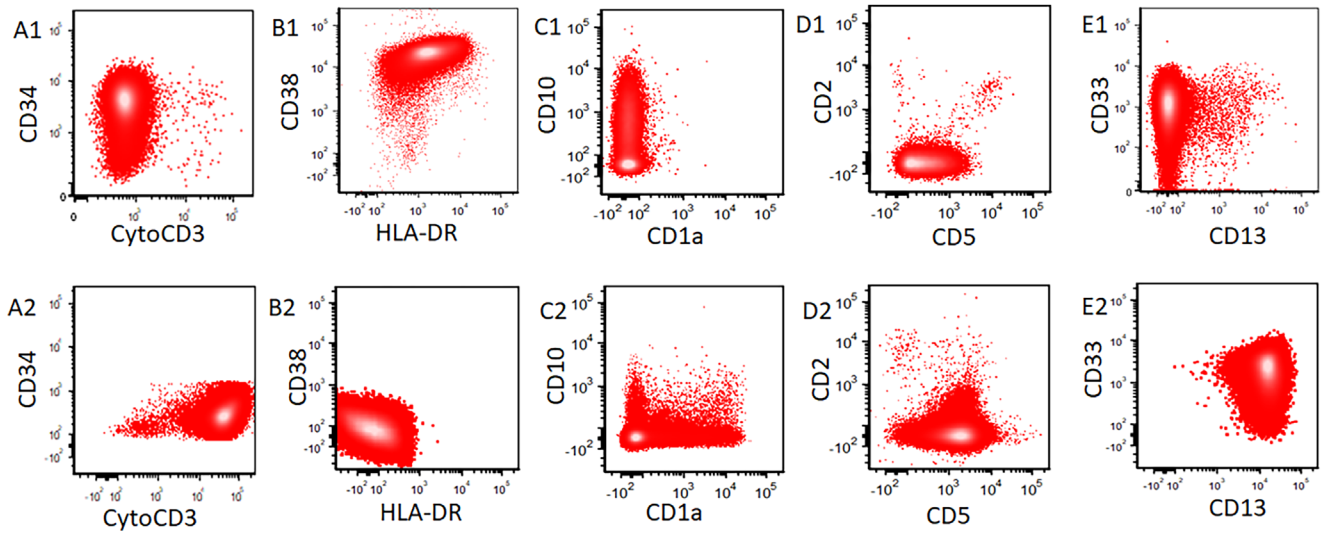

FIGURE 2 | Comparison of flow cytometric immunophenotypic features of neoplastic cells in bone marrow and medistainal mass. (A1-E1) The blasts in the bone marrow are positive for CD34, CD33, CD38, HLA-DR, and CD10 (partial) and are negative for CD1a, CD2, cytoCD3, CD5, and CD13. (A2–E2) The neoplastic cells in mediastinum mass are positive for CD1a (partial), cytoCD3, CD5, CD13, and CD33 and are negative for CD2, CD10, CD34, CD38, and HLA-DR.

Author Contributions

JX, FZJ, and SAW made the diagnosis. GT, SL, and HYW contributed to the cytogenetic and molecular studies. QW and JX wrote the manuscript. SYL, PL, and LJM edited the manuscript.

Ethics Statement

The study was performed in accordance with the principles of the Declaration of Helsinki and the institutional guidelines.

Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Data Availability Statement

No datasets were generated in this study.

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