# Emerging Necessity of Myeloid Mutational Analysis in Early T-cell Precursor Acute Lymphoblastic Leukemia/Lymphoma (ETP-ALL)

## Erken T-hücre Öncü Akut Lenfoblastik Lösemi/Lenfoma (ETP-ALL)'da Ortaya Çıkan, Miyeloid Mutasyon Analizi Gerekliliği

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**To the Editor,**

Early T-cell precursor (ETP) acute lymphoblastic leukemia/lymphoma (ETP-ALL) is a unique subtype of T-lymphoblastic leukemia (T-ALL) characterized by its distinct immunophenotypic profile and genetic signature. First described in 2009 as a type of T-ALL derived from thymic cells at the ETP differentiation stage [1], ETP-ALL was officially recognized as a distinct provisional entity by the 2017 World Health Organization classification [2]. The leukemic cells in ETP-ALL are committed to the T-cell lineage, but not irreversibly, and hence they retain the potential for myeloid/dendritic cell differentiation. This results in a distinct immunophenotypic profile characterized by positivity for T-cell antigens such as CD7 and cytoplasmic CD3, but also myeloid/stem cell antigens such as CD34, CD117, HLA-DR, CD13, CD33, and CD11b. By definition, the cells lack CD1a, C8, and MPO, whereas CD5 is either negative or only expressed in a minor subset of cells [2,3]. This typical immunophenotypic profile has been shown to be highly specific for the ETP-ALL gene expression signature, which, not surprisingly, shows a higher frequency of mutations associated with myeloid neoplasms such as *FLT3*, *DNMT3A*, and *IDH1/2* and a relatively lower frequency of more typical T-ALL not otherwise specified (T-ALL, NOS)-defining genetic lesions such as *NOTCH1* and *CDKN1/2* mutations [2]. Additionally, the 2022 International Consensus Classification recognized the disruption of the *BCL11B* locus as a highly sensitive and reliable diagnostic variable in favor of ETP-ALL compared to T-ALL-NOS [4]. Despite these significant recent advances in understanding this disease's molecular basis, the optimal therapeutic approach is poorly characterized and overall survival remains dismal. The high frequency of myeloid neoplasia-associated mutations in ETP-ALL presents a promising therapeutic window, however, and novel targeted therapies may help improve overall survival in the near future. We present the case of a 58-year-old man with *IDH1*-mutated ETP-ALL who attained prolonged and sustained remission with IDH1 inhibitor therapy given in combination with standard chemotherapy.

A 58-year-old man with no significant past medical history presented with a 6-week history of fatigue, bilateral lower extremity weakness, chills, and loss of appetite. A complete blood count revealed normocytic anemia (hemoglobin: 8.8 g/dL), absolute neutropenia (absolute neutrophil count: 1100/µL), and thrombocytopenia (platelet count: 122,000/µL). Morphological review of the peripheral blood smear revealed 15% circulating blasts, which were intermediate to large with irregular nuclear contours, fine chromatin, prominent nuclei, and a small amount of vacuolated basophilic cytoplasm (Figure 1A, black arrow). This prompted a bone marrow biopsy that revealed 73% blasts forming diffuse sheets in the bone marrow (Figure 1B) and markedly decreased background trilineage hematopoiesis. Immunohistochemistry performed on the bone marrow core biopsy sample showed that the blasts were diffusely positive for CD7 (Figure 1C), with a subset positive for CD34 and CD3, and negative for CD1a and TDT immunohistochemical stains. Flow cytometric analysis performed on the bone marrow aspirate sample revealed 70% blasts that were dim CD45+, surface CD3-, cytoplasmic CD3+, CD2-, predominantly CD7+ (85%), predominantly CD5- (variable/dim expression in 10%), CD4-, CD8-, CD16-, CD56-, CD57-, TDT-, CD34+, CD117-, HLA-DR+, CD33+, CD13-, CD14-, CD64-, cytoplasmic MPO-, CD19-, CD10-, kappa/lambda surface light chains-, and cytoplasmic CD79a- (Figure 1D). In light of these findings, a diagnosis of ETP-ALL was made. Chromosomal analysis showed a normal karyotype (46,XY). Fluorescence in situ hybridization testing for a T-lymphoblastic leukemia panel was negative for all probes, including -9p21 (*CDKN2A*), t(9;22) *ABL1/BCR* fusion, 11q23 (*MLL*), -17p13.1 (*TP53*), 7q34 (*TRB*), 14q11.2 (*TRAD*), t(10:11) *MLLT10/PICALM* fusion, and 1p33 (*TAL1/STIL*).

Given the increasing scientific evidence for a high prevalence of myeloid neoplasia (AML/MDS)-associated genetic mutations in ETP-ALL, we also performed comprehensive next-generation sequencing myeloid panel analysis of the bone marrow aspirate sample. The panel of 47 genes detected an isolated *IDH1*: c.394C>T; p.Arg132Cys pathogenic mutation with a variant allele frequency (VAF) of 42% and was negative for all other genes tested, including *FLT3*, *DNMT3A*, *TET2*, *KRAS*, and *NRAS*. The patient was treated with hyper-CVAD

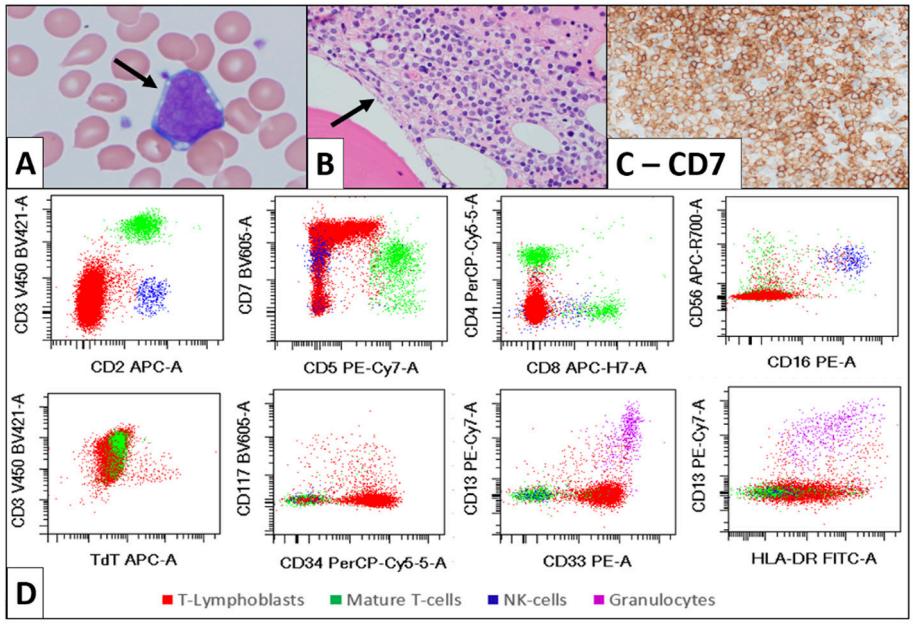


**Figure 1.** A) Peripheral blood smear revealed 15% circulating blasts, which were intermediate to large with irregular nuclear contours, fine chromatin, prominent nuclei, and a small amount of vacuolated basophilic cytoplasm (black arrow). B) Bone marrow biopsy revealed 73% blasts forming diffuse sheets in the bone marrow (black arrow). C) Immunohistochemical analysis of the bone marrow core biopsy sample showed blasts diffusely positive for CD7. D) Flow cytometric analysis of bone marrow aspirate sample revealed 70% blasts that were CD2-, predominantly CD5-, CD8-, CD16-, TDT-, CD34+, CD33+, and HLA-DR+.

(hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) induction therapy. A bone marrow biopsy after two cycles revealed no residual disease by morphology or flow cytometry; however, quantitative *IDH1* analysis by droplet digital polymerase chain reaction (ddPCR) was positive at 5.13% (mutated/total *IDH1*). The patient was treated with an additional two cycles of hyper-CVAD with the addition of an IDH1 inhibitor (ivosidenib). A repeat bone marrow analysis following the fourth cycle detected no residual disease by morphology or flow cytometry, and ddPCR was negative for mutated *IDH1* (sensitivity: 0.5%). This was followed by an allogeneic hematopoietic stem cell transplant. The patient continues to be in remission 26 months following the diagnosis. While it is not possible to ascertain whether the prolonged and sustained remission was due to the additional two cycles of hyper-CVAD or to IDH1 inhibition, or both, the therapeutic inhibition of a disease-driving mutation and the subsequent molecular remission as shown by high-sensitivity ddPCR indicates that the IDH inhibitor most probably played some therapeutic role in this case.

Despite the significant recent advances in our understanding of the genetic background of ETP-ALL, the optimal treatment regimen remains uncertain. ETP-ALL is a rare entity accounting for approximately 16% of childhood and 22% of all adult T-ALLs [5,6]. That rarity, combined with the fact that it is still a relatively newly described entity, has resulted in a lack of large-scale investigational studies exploring the optimal therapeutic approach. Although the genetic landscape of ETP-ALL is very heterogeneous and does not show a single unifying genetic lesion, the differences between ETP-ALL and T-ALL are quite striking and may, in the near future, open doors to novel therapies. Genetically and immunophenotypically, ETP-ALL sits at the crossroads of myeloid neoplasms (AML/MDS) and T-ALL, NOS. It is enriched in mutations that are typically more defining of myeloid neoplasia, including activating mutations driving RAS and cytokine receptor/JAK-STAT signaling (i.e., *FLT3, IGFR1*, *JAK1*, *JAK3*, *KRAS*, *NRAS*), DNA methylation genes (i.e., *IDH1/2*, *DNMT3A*, *TET2*), histone-modifying genes (i.e., *EED*, *SUZ12*, *EZH2*), and genes regulating hematopoietic development (i.e., *RUNX1*, *IKZF1*, *ETV6*, *GATA3*, *EP300*). In contrast, the frequency of mutations in genes commonly involved in the pathogenesis of T-ALL (i.e., *NOTCH1*, *CDKN1/2*, *FBXW7*) is substantially lower [7,8]. Our case highlights the importance of myeloid mutational analysis in ETP-ALL, which may detect a potential therapeutic target such as *IDH*, *FLT3*, or *EZH2.* This may help improve the prognosis of this otherwise dismal disease in the near future as well as provide a viable biomarker for molecular residual disease monitoring. In conclusion, testing for AML/MDS-associated genes should be considered in all cases of ETP-ALL, and the addition of targeted therapies might improve the poor outcome of ETP-ALL.

**Keywords:** T-lymphoblastic leukemia, Early T-cell precursor acute lymphoblastic leukemia/lymphoma, Myeloid, Next-generation sequencing, *IDH*, Molecular

**Anahtar Sözcükler:** T-lenfoblastik lösemi, Erken T-hücre öncü akut lenfoblastik lösemi/lenfoma, Miyeloid, Yeni-nesil dizileme, *IDH*, Moleküler

### Ethics

### Authorship Contributions

Concept- H.T., S.S.; Design- H.T., S.S.; Data Collection or Processing- H.T., S.S..; Analysis or Interpretation- H.T., S.S..; Literature Search- H.T., S.S.; Writing- H.T.

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