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# On the ridge between RUNX1 mutated ETP-ALL and AML: A Relapse-Driven Lineage Switch

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**Case Report**

**Keywords:** Lineage switch, acute myeloid leukemia (AML), multilineage dysplasia, early T-precursor lymphoblastic leukemia (ETP-ALL)

**Posted Date:** November 19th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-11564945/v1

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**Additional Declarations:** No competing interests reported.

# Abstract

Acute leukemia cases presenting a differentiation arrest at the T/myeloid interface are challenging to accurately diagnose and manage. Herein, we report the highly unusual case of a 66-year-old man who was initially diagnosed with acute myeloid leukemia (AML) with myelodysplasia related changes. After achieving remission following genoidentical allogeneic stem cell transplantation, he relapsed nine years later with an Early T-cell precursor lymphoblastic leukemia (ETP-ALL) showing strikingly similar morphologic, phenotypic and molecular features. The relapsed disease responded to azacytidine and venetoclax combination. This case underlines the overlap between ETP-ALL and AML from both diagnosis and clinical management points of view.


# Introduction

Early T-cell precursor lymphoblastic leukemia (ETP-ALL) is defined by an immunophenotype with absent mature T-cell markers, weak or negative CD5, and expression of stem cell (e.g., CD34, HLA-DR) and/or myeloid markers (e.g., CD11b, CD13, CD33, CD117). In fact, ETP-ALL blasts derive from the malignant counterpart of the earliest T-cell precursors that retain the ability to differentiate into both T-cell and myeloid lineages. This immaturity parallels with an 'ETP-like' transcriptomic signature with a higher stemness index [1]. This occasions frequent phenotypic overlap with T/myeloid mixed-phenotype acute leukemia (MPAL-T/M). Similarly, ETP-ALL harbors some molecular features typically found in acute myeloid leukemia (AML), together with less frequent *NOTCH1* mutations than in other T-ALLs [2]. In particular, a higher proportion of *RUNX1* mutations have been identified in the most undifferentiated malignant cells, arising from lymphoid-oriented early-stage progenitors in both AML and ETP-ALL [3].

Here we present an unusual case of an AML with myelodysplasia related changes (MRC), which subsequently relapsed into ETP-ALL while conserving its main morphological, immunophenotypic and molecular features, including high allele frequency *RUNX1* mutation. We aim to illustrate how the usual classification and treatment recommendations do not always account for the particular oncogenesis of early progenitor derived leukaemias.

Case Presentation

We report the case of a 66-year-old man without prior medical history. At first presentation, the patient exhibited bicytopenia (hemoglobin 6.4 g/dL, platelets 98 G/L) and 5% circulating blasts on the blood smear. Bone marrow examination revealed an 80% blast infiltration with a myeloid appearance (Fig. 1A), 28% of the blasts being myeloperoxidase (MPO) positive (Fig. 1B). A multilineage dysplasia was noted with an excess of ring sideroblasts (Fig. 1C). All of the aforementioned morphological features were consistent with AML-MRC, in accordance with the 2008 WHO classification.

Flow cytometry identified the blasts as CD34 + CD38- HLA-DR+ cells, co-expressing myeloid markers (CD117, CD33, CD13, MPO), and aberrant T-cell antigens (CD5 and CD7), but lacking cCD3. These findings confirmed the AML diagnosis. The karyotype was normal.

Intensive induction chemotherapy combining idarubicin and cytarabine was initiated. A genoidentical stem-cell transplant was performed following a reduced-intensity conditioning regimen. The patient achieved complete remission, confirmed by *WT1*-based minimal residual disease negativity one-month post-transplant and full donor chimerism at three months.

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Remarkably, nine years later, the patient presented with mild anemia (11.2 g/dL), thrombocytopenia (76 G/L), and 31% circulating blasts. Bone marrow examination showed 50% blast infiltration, harboring similar morphology to that at diagnosis, with signs of myeloid dysplasia (Figure 1 D), but no MPO detected by cytochemical staining (Figure 1E)**.** The relapse blasts, identified as CD34+ CD38+ HLA-DR+ cells, displayed a similar profile, with a broader expression of most stem and myeloid markers CD34, CD38, CD117, and CD33, contrasting with a smaller proportion of CD13 positivity (Figure 2A), but no MPO expression. Strikingly, CD5 and CD7 T-lineage markers persisted (albeit <75%, excluding the diagnosis of a near-ETP-ALL) compared to the initial diagnosis. The absence of MPO along with the new positivity of cCD3 supported the diagnosis of ETP-ALL.

High-throughput sequencing was performed on both the initial sample, cryopreserved after DNA extraction, and the relapse sample to compare the molecular profiles (Figure 2B). Interestingly, the mutations *RUNX1* p.Ser322*, *SF3B1* p.Lys666Thr, *ASXL2* p.Gln699fs and one subclonal mutation in *NRAS* gene p.Gly13Asp, were identified with high allele frequency on both time points. The relapse molecular profile was more complex comprising multiple additional *NRAS, KRAS* and *PTPN11* mutations. Of note, the chimerism analysis showed an 80% recipient profile after relapse, confirming graft rejection. Altogether, the targeted sequencing analysis supported relapse over *de novo* malignancy.

The patient was managed according to guidelines for ALL treatment in older patients. Following induction failure, drug screening suggested sensitivity to azacytidine and venetoclax therapy, which were administered alongside treatment of meningeal involvement through lumbar punctures and asparaginase. This regimen progressively reduced circulating blasts, leading to cytological remission two months after relapse. The patient subsequently died from cardiac complications at the age of 75.

## Discussion

ETP-ALL is a particularly challenging diagnosis given the important overlap with AML or MPAL, particularly when presenting features of an AML-MRC. This underscores the importance of employing a comprehensive range of diagnostic techniques, including morphology, cytometry, and cytochemistry, as the current thresholds outlined in both the WHO and ICC 2022 remain contentious and do not always facilitate definitive classification.

This unusual lineage switch from AML-MRC to ETP-ALL underlines the immature phenotype characteristic of ETP-ALL. Lambo and colleagues described a similar case and mapped the transcriptomic profile for diagnosis and relapse samples, showing a clustering into both pro-B, multilineage and granulo-monocytic progenitors [4]. In the present case, the persistence of multilineage dysplasia following relapse, together with the high allele frequency contrasting with the moderate blast percentage, suggests that leukemic mutations may have penetrated myeloid progenitors, consistent with a non-committed blast profile.

The treatment of ETP-ALL generally relies on ALL-like regimens. However, given the rarity of these diseases, especially in adults, there is a lack of prospective data to guide therapy. In our case, even if a rigorous comparison is impossible, the patient appeared to respond more favorably to AML-like regimens, as evidenced by the initial positive response and the sensitivity to azacytidine and venetoclax following relapse. By contrast, the protocol induction failed to control the circulating blast percentage. This is consistent with the description of a subgroup of ETP-ALL with poor primary response, DNA hypermethylation on epigenetic analysis, and sensitivity to hypomethylation agents using a xenograft model [5]. Taken together, the wide heterogeneity of the interface leukemias and their biological complexity highlight the value of drug testing, which in this case ultimately facilitated cytological remission.

## Conclusion

We describe a highly unusual case of delayed AML-MRC relapse accompanied by a lineage switch, ultimately leading to reclassification as ETP-ALL. This case further supports the notion that ETP-ALL is biologically distinct from other T-ALL subtypes and raises important considerations for both diagnosis and clinical management. In fact, the phenotypic and molecular similarities with AML, along with the

high relapse rate characteristic of ETP-ALL, support the exploration of therapeutic approaches that incorporate AML-oriented treatments.

## Declarations

# Funding Statement

The authors did not receive any specific funding to conduct this study, as the clinical case reported here was part of the routine hospital activities of the laboratories and the clinical department.

Conflict of Interest Disclosure

The authors confirm the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Approval Statement

Not applicable.

Patient Consent Statement

The data presented in this case report are limited to clinical and biological data collected as part of the patient's care, for which consent was obtained in accordance with applicable regulations. Additionally, written informed consent for participation in a genetic research study was obtained from the patient during their lifetime. No separate written informed consent specifically for publication of this case report was obtained. The patient had died before manuscript preparation.

Permission To Reproduce Material from Other Sources

Not applicable.

Clinical Trial Registration

Not applicable.

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Author Contribution

M.Y.S. and N. K.: collected the biological and clinical data and provided a first draft of the manuscript; D.D., B.B. and N.F.: analysed morphological, cytochemical and flow cytometry data at diagnosis, follow up and relapse; S.T. and V.T.Q.: analysed molecular data at diagnosis, follow up and relapse; P.C. and P.V: managed the patient; I.S.: critically reviewed the manuscript; L.C.: performed drug screening analysis and provided valuable insight for literature review; O.W.B.: made the study possible by coordinating collaboration among laboratories and the clinical department, and provided valuable guidance on the manuscript writing; B.S.: formatted the figures, finalized the manuscript's initial draft and completed the literature review.

# Acknowledgement

The authors acknowledge the AP-HP University hospitals for their framework that fosters cutting-edge medicine and research, together with the strong cooperation of various clinical and laboratory departments permitted by its structural support. Similar projects are made possible thanks to the strong ties established between research and hospital teams.ChatGPT (OpenAI, San Francisco, CA, USA) was occasionally used to assist with language editing and improving clarity of the manuscript. All content was reviewed and approved by the authors, who take full responsibility for the final version.

# Data Availability

The authors have reported their data as transparently as possible in the manuscript. Given the brevity of the article and due to difficulties with anonymization, the raw data have not been provided directly. All molecular data, flow cytometry data, or supplementary images not included in the manuscript are available upon request.

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## Figures

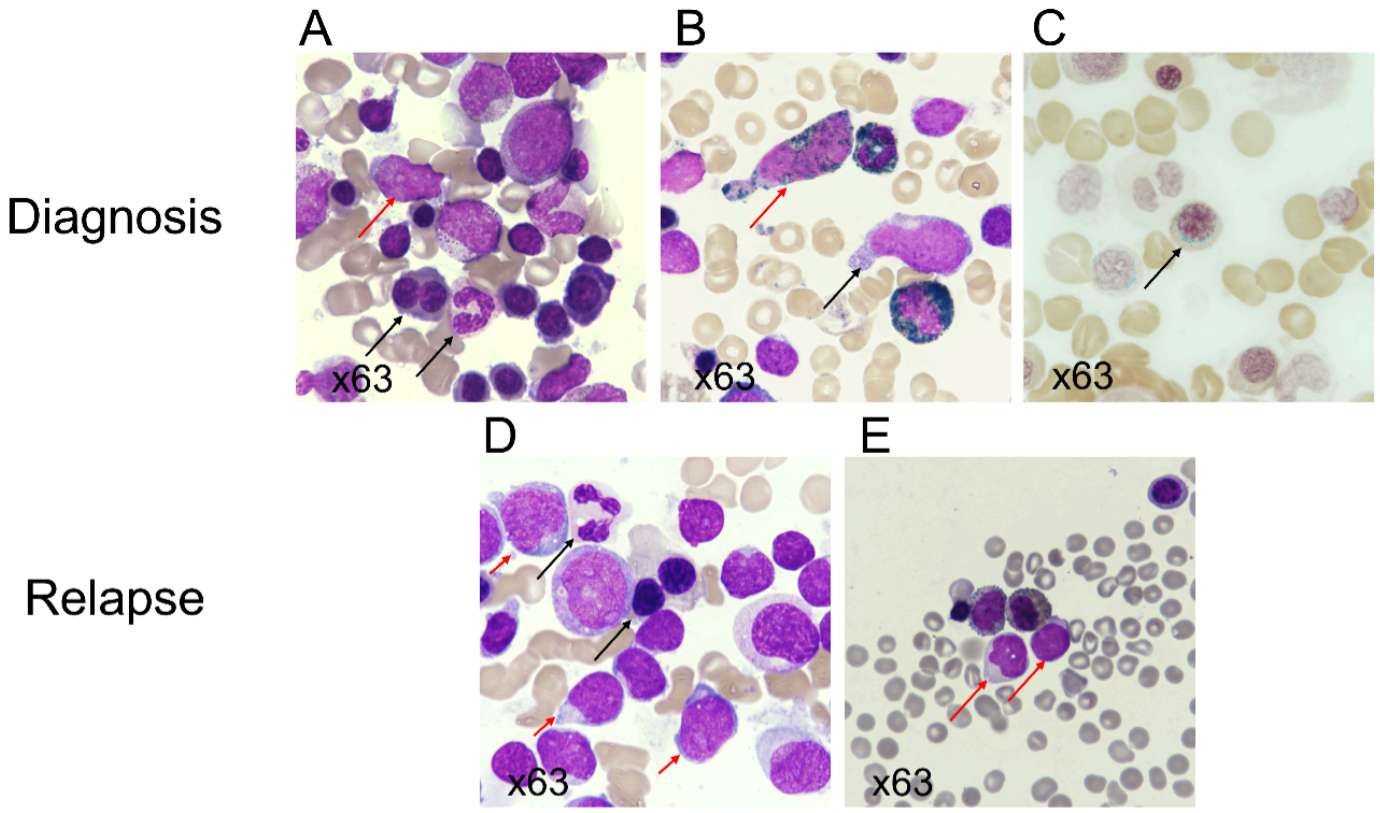



**Figure 1**

Morphological evaluation of bone marrow at diagnosis after staining. The objectives used is given in the bottom left corner of each image. A) Bone marrow blasts (red arrows) and multilineage dysplasia (black arrows) observed after May-Grünwald Giemsa (MGG) staining at diagnosis. B) Blasts observed after intracytoplasmic MPO cytochemistry staining using benzidine showing both positive (red arrow) and negative blasts (black arrow) at diagnosis. C) Perls Prussian Blue stain, positive for ring sideroblasts (black arrow) at diagnosis, evaluated at 32%. D) Bone marrow blasts (red arrows) and multilineage dysplasia (black arrows) observed after MGG staining at relapse. E) Blasts observed after intracytoplasmic MPO cytochemistry staining using benzidine showing a complete negativity (red arrows) at relapse.

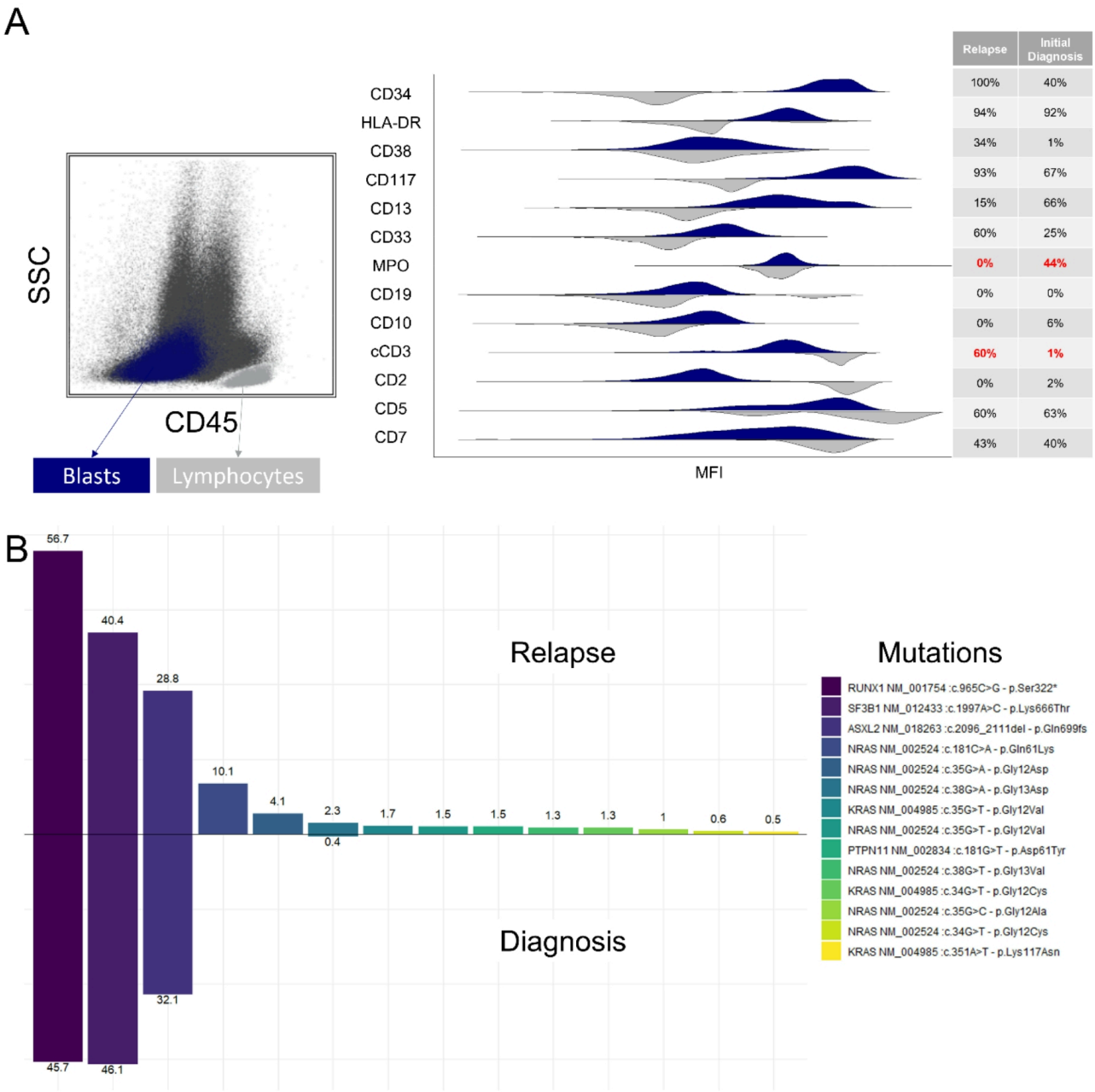



**Figure 2**

Phenotypic and molecular features of bone marrow blasts at relapse compared to the diagnosis. A) Side scatter against CD45 diagram highlighting both blasts (blue dots) and lymphocytes (grey dots) used as controls for marker positivity (left panel). Mean Fluorescence Intensity (MFI) for each main lineage markers represented as density plots for blasts (in blue) compared to lymphocytes used as control population (in grey) for the relapse sample (medium panel). The percentage of positive cells for each marker at relapse and initial diagnosis is reported in the adjacent table (right panel). The contrasting markers changing the lineage identity are flagged in red. B) Allele frequencies (in %) of all mutations

identified by high-throughput sequencing at relapse (top) and diagnosis (down), with barplots ordered by allele frequency at relapse. Allele frequencies are annotated above (relapse) or below (diagnosis) each bar. Mutations are listed in the legend on the right and color-coded using a gradient.