

Bilineal evolution of a *U2AF1*-mutated clone associated with acquisition of distinct secondary mutations

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Key Points

- We report a bilineal process with truncal *U2AF1* mutation and lineage-specific secondary mutations in B-lymphoblast and myeloid populations.
- This case highlights an atypical mechanism of BLL leukemogenesis and illustrates the potential advantage of fractionated sequencing.

Rare hematologic malignancies display evidence of both myeloid and lymphoid differentiation. Here, we describe such a novel bilineal event discovered in an adult woman with B-lymphoblastic leukemia (BLL). At the time of BLL diagnosis, the patient had a normal karyotype and a bulk sequencing panel identified pathogenic variants in *BCOR*, *EZH2*, *RUNX1*, and *U2AF1*, a genotype more typical of myeloid neoplasia. Additionally, the patient was noted to have 3-year history of cytopenias, and morphologic dyspoiesis was noted on post-treatment samples, raising the possibility of an antecedent hematologic disorder. To investigate the clonal architecture of her disease, we performed targeted sequencing on fractionated samples enriched for either B-lymphoblasts or circulating granulocytes. These studies revealed a truncal founder mutation in the spliceosome gene *U2AF1* in both fractions, while distinct secondary mutations were present only in B-lymphoblasts (*BCOR*, *NRAS*) or myeloid cells (*ASXL1*, *EZH2*, *RUNX1*). These results indicate that both processes evolved from a common *U2AF1*-mutated precursor, which then acquired additional mutations during a process of divergent evolution and bilineal differentiation. Our findings highlight an atypical mechanism of BLL leukemogenesis and demonstrate the potential utility of fractionated sequencing in the characterization of acute leukemia.

Introduction

Hematologic malignancies are typically characterized by restricted myeloid or lymphoid differentiation. Less frequently, neoplasms show evidence of bilineal differentiation, reflecting a neoplastic stem cell population that retains the ability to differentiate along both lineages. For instance, rare acute leukemias of ambiguous lineage may show bilineal differentiation, with distinct lymphoid and myeloid blast populations.^{1,2} Similarly, a small subset of patients with chronic myeloid leukemia develop a B-lymphoblast or bilineal blast crisis, supporting the presence of a primitive *BCR-ABL1*⁺ clone that retains lineage plasticity.³ In contrast, lymphoblast crisis has been described only rarely in other chronic myeloid neoplasms, like myelodysplastic syndrome (MDS),⁴⁻⁶ and to our knowledge, there are no reports comparing the mutational patterns in the lymphoblast and myeloid branches in such cases. Here, we report a patient with B-lymphoblastic leukemia and a concurrent MDS-like myeloid clone, which evolved from a common *U2AF1*-mutated precursor through acquisition of distinct secondary mutations.

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Table 1. Mutational findings in bulk, CD19-enriched, and CD33-enriched samples

Mutation	VAF, %		
	Bulk sample	CD19 fraction	CD33 fraction
<i>ASXL1</i> R693*	Not tested	Not detected	51
<i>BCOR</i> S499fs	29	47	Not detected
<i>EZH2</i> R614I	6	Not detected	32
<i>NRAS</i> Q61L	15	22	Not detected
<i>RUNX1</i> S322*	5	Not detected	33
<i>U2AF1</i> Q157P	43	49	49

Not detected = to a limit of detection of 3% variant allele fraction (VAF).

Case description

A 75-year-old woman was evaluated for a 3-year history of anemia (hemoglobin, 8.1 g/dL; mean corpuscular volume, 95.3 fL) and a 14-month history of thrombocytopenia (platelets, $86 \times 10^9/L$). Bone marrow biopsy confirmed a diagnosis of acute leukemia, with 64% blasts by manual aspirate differential. Morphologic assessment of other lineages was limited in the bone marrow aspirate, but subtle dysgranulopoiesis and dyserythropoiesis were noted. By flow

cytometry, the blasts expressed CD9, CD10, CD19, CD22, CD24, CD38, CD99, HLA-DR, and terminal deoxynucleotidyltransferase (TdT), with aberrant coexpression of CD7, CD11b, CD13, CD117, and CD123. Other analyzed markers were negative, including lineage-defining T-cell (CD3) and myeloid antigens (myeloperoxidase [MPO]), CD33, and analyzed monocytic antigens (CD14, CD36, and CD64). MPO was also negative by immunohistochemistry. Given coexpression of CD10, CD19, and CD22 and absence of lineage-defining T-cell or myeloid markers, a diagnosis of B-lymphoblastic leukemia (BLL) was rendered per 2016 World Health Organization criteria.² This interpretation was also supported using the European Group for the Immunological Classification of Leukemias scoring system, which recognizes lineage based on a phenotypic score >2 (B-lineage score, 4.5 because of CD22, CD10, CD19, and TdT expression; myeloid score, 2.0 because of CD13 and CD117 expression; T-lineage score, 1.0 because of TdT and CD7 expression).⁷

Unexpectedly, however, a 140-gene next-generation sequencing (NGS) panel identified a mutational profile more typical of myeloid malignancies, including pathogenic variants in *BCOR*, *EZH2*, *RUNX1*, and *U2AF1* (Table 1). Conventional cytogenetic studies revealed a normal karyotype, but interphase fluorescence in situ

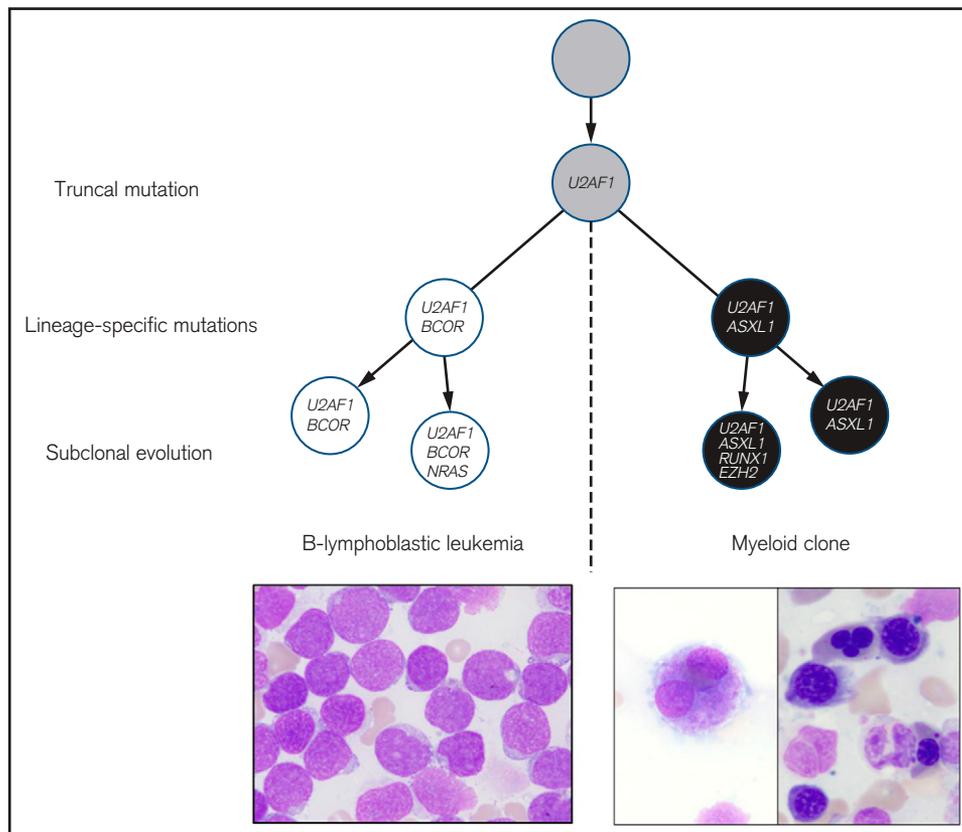


Figure 1. Branched evolution of a *U2AF1*-mutated progenitor to distinct B-lymphoblast and myeloid clones. Truncal mutation: Initially, a *U2AF1* mutation is proposed to have arisen in a lineage-uncommitted progenitor. Lineage-specific evolution: Subsequently, separate subclones acquired *BCOR* and *ASXL1* mutations, which appeared lineage restricted. Subclonal evolution within a lineage: Finally, the B-lymphoblast and myeloid clones continued to evolve by acquisition of additional subclonal mutations. Morphologic findings of increased blasts after initial minihyper CVD (lower left, 600× original magnification) and morphologic dyspoiesis after 2 cycles of inotuzumab are shown. Dysplastic features included megakaryocytes with abnormal lobation (middle image, 600× original magnification) and erythroid multinuclearity, nuclear irregularity, and nuclear fragmentation (right, 2100× total magnification). All microscopic images represent May Grunwald-Giemsa stains.

hybridization analysis with multiple probes suggested the presence of a nondividing hyperdiploid or tetraploid clone.

Because of patient age and comorbidities, treatment was initiated with a low-intensity regimen of cyclophosphamide, vincristine, and dexamethasone (mini-hyper-CVD). Bone marrow aspirates performed after the first and second cycles showed persistent disease, with 50% to 52% B-lymphoblasts by manual aspirate differential (Figure 1 bottom left). Although comprehensive immunophenotyping was not performed in follow-up samples, the leukemia continued to express CD10, CD19, and CD22 by flow cytometry and remained negative for MPO by both cytochemical stain and immunohistochemistry. Importantly, there was no increase in immature myeloid cells. Rather, granulocytic and erythroid precursors were relatively decreased in number but exhibited a full spectrum of maturation. Trilineage atypia, although present, was difficult to interpret in the setting of ongoing cytotoxic chemotherapy. Conventional cytogenetic studies confirmed the presence of a near-tetraploid karyotype (91,XXXX,-18).

Because of persistent BLL, the patient was transitioned to inotuzumab. After 2 cycles of CD22-targeted monotherapy, her bone marrow blast count was reduced to 6%. However, she remained anemic (hemoglobin, 9.2 g/dL) and thrombocytopenic (platelets, $90 \times 10^9/L$). Morphologic review of the peripheral blood and bone marrow showed trilineage myeloid dyspoiesis, including hypogranular and pelgeroid neutrophils, hypolobated megakaryocytes, and atypical erythroid precursors (Figure 1 bottom right). Given the mutational profile of the patient's disease at diagnosis, the long antecedent history of cytopenias, and persistent morphologic dyspoiesis on inotuzumab, we sought to determine whether she might have concurrent or even related B-lymphoblast and myeloid clones.

Methods

Enrichment of B-lymphoblast and myeloid populations

B-lymphoblasts were enriched from a bone marrow sample (after first cycle of mini-hyper-CVD) with ~50% B lymphoblast preenrichment using an EasySep Pan-B Cell Enrichment Kit on an Easy-8 Magnets (Stemcell Technologies, Vancouver, BC, Canada). Flow cytometry confirmed enrichment to ~90% CD19⁺ cells. CD33⁺ myeloid populations were enriched from a concomitant peripheral blood sample with <1% B-lymphoblasts using an Easy-Sep HLA Chimerism Whole Blood CD33-Positive Selection Kit on a RoboSep Automated Cell Separator (Stemcell Technologies). Postenrichment flow cytometry confirmed >90% CD33⁺ cells.

NGS panels

Bulk sequencing of the diagnostic sample was performed using a custom 140-gene amplicon-based NGS panel (Fluidigm, San Francisco, CA; panel design by PathGroup, Nashville, TN). After library preparation, sequencing was performed on an Illumina NextSeq instrument (Illumina, San Diego, CA).

Sequencing of the posttreatment B-lineage and myeloid-enriched cell populations was performed using a customized 35-gene capture-based NGS panel (SOPHiA Genetics, Lausanne, Switzerland; panel design by University of North Carolina Health, Chapel

Hill, NC). Sequencing was performed on an Illumina MiSeq instrument.

Results and discussion

To investigate the clonal architecture of this process, we performed targeted NGS after B-lineage and myeloid-lineage bead enrichment in bone marrow and peripheral blood samples, which were collected after the first cycle of mini-hyper CVD. After initial therapy, the previously reported *U2AF1* Q157P mutation was detected in both the B-lymphoblast fraction (VAF, 49%) and in the myeloid fraction (VAF, 49%), suggesting a shared clonal origin (Table 1). Previously reported *BCOR* and *NRAS* mutations were detected only in the B-lymphoblast fraction. In contrast, the myeloid fraction was positive for the previously reported *RUNX1* and *EZH2* mutations, as well as an *ASXL1* R693* mutation, which was not covered in the diagnostic NGS panel.

Identification of a common *U2AF1* mutation in both B-lymphoblast- and myeloid-enriched fractions supports a shared clonal origin of these 2 populations. Conceding some inherent limitations of bulk sequencing analysis in delineating clonal architecture,⁸ the observed ~50% VAF in each fraction suggests a heterozygous mutation in essentially all cells in both populations. These results support a model in which the *U2AF1* mutation arose as a truncal mutation in a lineage-uncommitted progenitor cell (Figure 1). Subsequently, subclonal mutations were acquired in *BCOR* and *ASXL1* in a lineage-specific fashion. Finally, from the observed VAFs, we infer that the B-lymphoblast and myeloid clones continued to evolve through acquisition of additional subclonal mutations.

Although mutational ontogeny can be inferred with the available results, the chronology of B-lymphoblast and myeloid clone evolution is more difficult to assess. Unfortunately, no archived samples were available to assess mutations before the patient's BLL diagnosis. However, in a patient with longstanding bicytopenias and at least mild multilineage morphologic dyspoiesis at diagnosis, identification of a high VAF myeloid clone suggests preexisting low-grade MDS.⁹ Notably, mutations in both the myeloid (*U2AF1*, *ASXL1*, and *EZH2*) and B-lymphoblast (*U2AF1* and *BCOR*) fractions have also been previously identified as molecular signatures of secondary leukemia in patients with acute myeloid leukemia.¹⁰ As such, this patient's BLL may be analogous to a B-lymphoblast crisis arising in the setting of a chronic myeloid neoplasm, via branched evolution of a common *U2AF1*-mutated precursor. However, we acknowledge that evaluation of dysplasia was limited in the diagnostic sample and is challenging in the setting of recent therapy.¹¹ As such, it is difficult to fully exclude alternative etiologies for the patient's cytopenias, as would be required to fulfill World Health Organization criteria for superimposed MDS.¹² Even so, the morphologic findings, longstanding cytopenias, and high VAF of the *U2AF1*, *ASXL1*, *RUNX1*, and *EZH2* mutations within the myeloid subclone all support the presence of at least an MDS-like myeloid clone.¹³

To our knowledge, there are no prior reports describing divergent mutational events in myeloid and B-lymphoblast lineages from a common precursor in a patient with BLL. In addition, *U2AF1* mutations are very rare in published series and databases of acute lymphoblastic leukemia, occurring, for instance, in 0.3% of BLLs and 1% of T-lymphoblastic leukemias in COSMIC.^{14–16} Interestingly, almost all reported *U2AF1* mutations in acute lymphoblastic

leukemia involve amino acid R35, rather than the myeloid mutation hotspots at positions S34, R156, and Q157, the latter of which was mutated in the present case. However, myeloid mutational profiles have been described in patients with therapy-related BLL,¹⁷ raising the possibility that similar processes may be underappreciated, particularly in older adults with lymphoblastic leukemia.

Importantly, our results do not provide functional evidence that these secondary mutations are directly responsible for driving B-lymphoblast and myeloid differentiation in this bilineal neoplasm.¹⁸ However, through B-lymphoblast and myeloid enrichment, we were able to use these mutations as molecular markers of a branching evolutionary process that was associated with bilineal differentiation. This case highlights not only an atypical mechanism of BLL leukemogenesis but also the advantage of fractionated sequencing, as compared with the bulk sequencing methods that are currently standard in clinical laboratories.

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Authorship

Contribution: N.D.M. designed the fractionation studies, interpreted the enriched sequencing studies, and was the primary author of the manuscript; J.G. and S.M.J. interpreted the follow-up bone marrow

biopsies and wrote portions of the text; L.C. assisted in interpretation of the enriched sequencing studies; E.T.W. performed the fractionation studies; P.K.C. interpreted the bulk sequencing study; J.F.Z., M.C.F., and T.Z. provided clinical care for the patient; T.B.A. provided consultative expertise on bilineage malignancies; and all authors critically reviewed the manuscript.

Conflict-of-interest disclosure: P.K.C. is employed by and has stock interest in PathGroup, unrelated to this work. J.F.Z. has received unrelated research funding from Arog, Astex, Gilead, Merck, Sumitomo Dainippon Pharma, and Takeda; honoraria for serving on advisory boards with Agios/Servier, Bristol-Myers Squibb, Daiichi Sankyo, Genentech, Gilead, Shattuck Labs, and Takeda; and consultancy funds from AbbVie and Takeda. M.F.C. has received unrelated research funding from Bellicum Pharmaceuticals, MacroGenics, and Rafael Pharmaceuticals and consultancy funds from Daiichi Sankyo, Agios, MacroGenics, and Ragael Pharmaceuticals. The remaining authors declare no competing financial interests.

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