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SHORT REPORT

From primary myelofibrosis to chronic myeloid leukemia, *BCR::ABL1*+ B-Lymphoblastic leukemia, and back to primary myelofibrosis: An illustration of dynamic clonal evolution

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Abstract

The simultaneous detection of *BCR::ABL1* and *JAK2* V617F was rarely reported and their clonal relationship and dynamic clonal shift were not characterized. Here, we described a unique case with the initial presentation as *JAK2* V617F+ primary myelofibrosis, followed by the emergence of *BCR::ABL1*+ chronic myeloid leukemia. The patient then developed *BCR::ABL1*+ B-lymphoblastic leukemia. Treatment for B-lymphoblastic leukemia prompted a regression to the state of primary myelofibrosis. In light of these observations, we proposed a clonal evolution model for this case.

KEYWORDS
BCR::ABL1 + B-ALL, clonal evolution, CML, *JAK2* V617F, PMF

1 | INTRODUCTION

BCR::ABL1 and *JAK2* V617F represent two distinct genetic drivers within the spectrum of myeloproliferative neoplasms (MPNs). Chronic myeloid leukemia (CML) is primarily driven by *BCR::ABL1* fusion, while Philadelphia chromosome-negative MPNs, including essential thrombocythemia, primary myelofibrosis (PMF), and polycythemia vera, are commonly associated with the *JAK2* V617F mutation. Although exceedingly rare, instances of co-existing *BCR::ABL1* and *JAK2* V617F have been documented in certain patients [1–3]. In these exceptional cases, the emergence of *BCR::ABL1* can precede, coincide with, or come after *JAK2* V617F. Despite previous reports of concurrent *BCR::ABL1* and *JAK2* V617F, the occurrence of blast transformation in such cases remains inadequately characterized, and the full extent of the underlying dynamic clonal evolution remains to be explored.

In this study, we present a unique case involving the sequential development of three hematologic malignancies. The patient's initial diagnosis was *JAK2* V617F-positive PMF. Two years later, CML harboring *BCR::ABL1* fusion emerged with the concurrent existing PMF. Subsequently, the patient encountered another disease progression, transforming to *BCR::ABL1*-positive B-lymphoblastic leukemia (B-ALL), occurring one year following the CML diagnosis. Notably, intensive chemotherapy administered for the B-ALL successfully diminished the *BCR::ABL1*-positive clone; however, the underlying PMF persisted. In light of comprehensive assessments of bone marrow morphology and the status of *JAK2* V617F and *BCR::ABL1*, we proposed a dynamic clonal evolution model that elucidates the intricate path of disease progression.

2 | CASE PRESENTATION

The patient is a 72-year-old man who initially presented with splenomegaly 43 months ago. The complete blood count (CBC) was as follows: a white blood cell (WBC) count of $9.0 \times 10^3/\mu$L with 67% neutrophils, hemoglobin (Hb) level of 12.1 g/dL, and platelet count of $153 \times 10^3/\mu$L. Review of the peripheral blood smear (Figure 1A) showed anemia with rare circulating nucleated red cells and moderate anisopoikilocytosis, including occasional teardrop cells. Bone marrow core biopsy (Figure 1B) showed a hypercellular (80%) marrow with

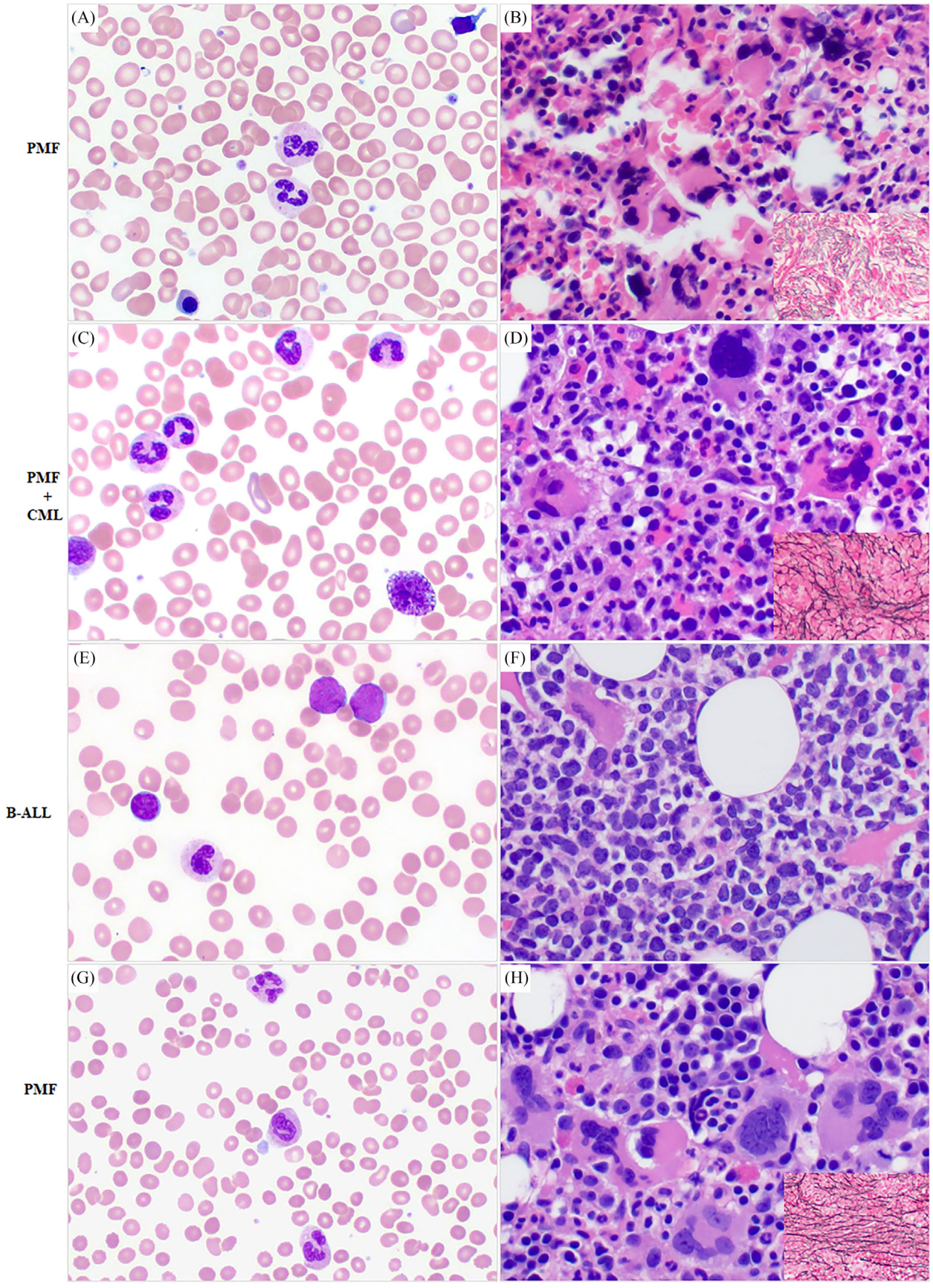

FIGURE 1 Peripheral blood (left) and bone marrow (right) findings at the different phases of disease. (A, B) Primary myelofibrosis (PMF); (C, D) PMF and chronic myeloid leukemia (CML); (E, F) B-lymphoblastic leukemia (B-ALL); (G, H) post-B-ALL (inset: reticulin stain).

granulocytic and megakaryocytic hyperplasia. Megakaryocytes formed dense clusters in some areas and they were variable in size and shape, with many being hyperchromatic. Background fibrosis is present. Reticulin stain (Figure 1B, inset) showed increased reticulin fibrosis, MF-2. Immunostains showed no increased blasts by CD34. Conventional karyotyping analysis showed a normal karyotype 46, XY, [20]. Fluorescence in situ hybridization (FISH) was negative for *BCR::ABL1*. A myeloid mutation panel by next generation sequencing showed *JAK2* V617F with a variant allele frequency (VAF) of 44.1%. A diagnosis of PMF was rendered and the patient was followed with regular blood tests and under observation.

Approximately 20 months after the initial diagnosis of PMF, the patient was found to have rising WBC counts. CBC study showed a WBC count of 44.8 $\times$ $10^3/\mu$L (77.2% neutrophils), Hb level of 10.5 g/dL, and platelet count of 141 $\times$ $10^3/\mu$L. Persistent splenomegaly (23 cm) was shown by CT scan. Peripheral blood smear (Figure 1C) showed neutrophilia without dysplasia. Basophils were increased in numbers, accounting for 3% of WBCs. A peripheral blood smear showed anemia with moderate anisopoikilocytosis and occasional circulating nucleated forms. No circulating blasts were identified. Bone marrow core biopsy (Figure 1D) showed a hypercellular (90%) marrow with granulocytic and megakaryocytic hyperplasia. Similar to the prior biopsy, megakaryocytes were increased and formed dense clusters in some areas. They were pleomorphic with some hyperchromatic forms. "Dwarf" megakaryocytes typically seen in CML were not obvious in the core biopsy. Reticulin stains showed persistent myelofibrosis, MF-2. *JAK2* V617F mutation was persistent with a VAF of 45.8%. Conventional karyotyping analysis showed the emergence of Philadelphia chromosome: 46, XY, t(9;22)(q34;q11.2)[20]. FISH study confirmed *BCR::ABL1* fusion, identified in 87% of cells. A b2a2 BCR::ABL1 fusion transcript coding for the 210 kDa fusion protein was detected by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). A diagnosis of concurrent PMF and CML was rendered. The patient started treatment with Dasatinib 50 mg daily. He showed hematologic improvement initially but had persistent disease at molecular level with the percentage of BCL-ABL1 to ABL1 transcript at 3.1% 5 months after therapy.

Approximately 12 months after the diagnosis of composite PMF and CML, the patient presented with marked leukocytosis, anemia, and thrombocytopenia. CBC study showed a WBC count of 223 $\times$ $10^3/\mu$L, a hemoglobin level of 8.8 g/dL, and a platelet count of 77 $\times$ $10^3/\mu$L. Multiple differential counts showed circulating blasts ranging from 43% to 55%. On blood smear (Figure 1E), blasts were intermediate sized with round to slightly irregular nuclei, dispersed chromatin, distinct nuclei and small amounts of basophilic agranular cytoplasm. Bone marrow core biopsy (Figure 1F) showed sheets of immature cells, consistent with blasts. Trilineage hematopoiesis was significantly decreased. Flow cytometric analysis showed blasts were B-lineage, positive for CD10, CD19, CD20, CD22, and TdT. They were negative for cytoplasmic CD3 and MPO. Conventional karyotyping analysis from peripheral blood showed 12 (60%) out of 20 cells with Philadelphia chromosome: 46,XY,t(3;12)(q26.3;q21),t(9;22)(q34;q11.2)[7]/46,idem,del(11)(q23)[3]/46,Y,t(X;4)(p22.3;q27),del(7)(p13),t(9;22)(q34;q11.2)[2]/46,XY[8]. FISH study performed on peripheral blood showed that 53% of the cells exhibited *BCR::ABL1* fusion and the fusion signal was identified in blasts only, not in segmented neutrophils. A b2a2 BCR-ABL1 fusion transcript coding for the 210 kDa BCR-ABL1 fusion protein was detected by quantitative real-time RT-PCR. Three ABL1 mutations were detected including p.T315I, p.F317L, and p.V299L. Mutational analysis showed persistent *JAK2* V617F mutation with a VAF of 41.2%. The overall findings were diagnostic for B-ALL. The patient was treated with mini-hyper-CVD (cyclophosphamide 150 mg/m^2 every 12 h for six doses, dexamethasone 20 mg per day, vincristine 2 mg per body, no anthracycline, methotrexate 250 mg/m^2, cytarabine 150 mg/m^2 every 12 h for four doses) with ponatinib 30 mg and blinatumomab. Methotrexate and cytarabine intrathecal injections were also performed. One month after the treatment for B-ALL, blood and bone marrow biopsies were performed for evaluation of response. CBC showed a WBC count of 13.6 $\times$ $10^3/\mu$L with 90% neutrophils, Hb level of 8.3 g/dL, and platelet count of 142 $\times$ $10^3/\mu$L. Peripheral blood smear (Figure 1G) showed neutrophilia with no circulating blasts. RBCs showed anemia with moderate anisopoikilocytosis. Bone marrow core biopsy (Figure 1H) showed a hypercellular (90%) marrow with granulocytic and megakaryocytic hyperplasia. Similar to pre-B-ALL bone marrows (Figure 1B,D), megakaryocytes were increased in numbers, forming clusters with some hyperplastic forms, which was consistent with PMF. Reticulin stain (Figure 1H, inset) showed grade 2 myelofibrosis (MF-2). Bone marrow smears and touch imprint showed no increased blasts. Flow cytometric analysis showed no immunophenotypic support for residual B-ALL. Conventional karyotyping analysis showed a normal male karyotype, 46,XY[8]. FISH was negative for BCR::ABL1. A b2a2 BCR-ABL1 fusion transcript coding for the 210 kDa BCR-ABL1 fusion protein was detected by quantitative real-time RT-PCR with a very low percentage of BCR-ABL1 to ABL1 transcripts of 0.05%. Overall, there was no morphologic or immunophenotypic support for B-ALL. The overall findings were consistent with persistent PMF. The patient received four cycles of chemotherapy with the addition of inotuzumab ozogamicin starting with the second cycle. The patient developed heart failure and the fourth cycle treatment is blinatumomab only.

At the last follow-up, 7 months after B-ALL diagnosis, the patient was in morphologic and immunophenotypic remission for B-ALL and CML. The most recent bone marrow biopsy showed persistent PMF (MF-2) without evidence of B-ALL. He is currently on cycle 1 of maintenance therapy with vincristine, prednisone, and ponatinib.

3 | DISCUSSION

Based on the bone marrow morphologic findings and the dynamic changes in *JAK2* V617F and *BCR::ABL1*, a clonal evolution model (diagram created using PyFish) was proposed (Figure 2) as the most plausible scenario for this particular case. Considering the frequently heterozygous nature of the *JAK2* V617F mutation, the 44.1% VAF observed upon the initial PMF diagnosis suggested that 80%–90% of cells carry this mutation. Although *BCR:ABL1* fusion was undetectable

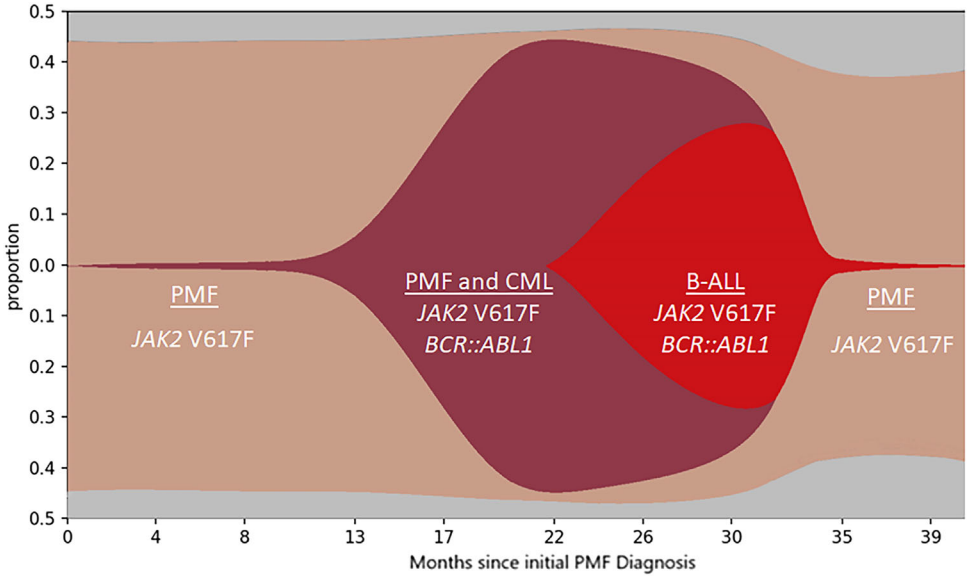

FIGURE 2 Proposed clonal evolution diagram. The left axis represents the proportion of cells exhibiting mutation(s), plotted against the timeline of months since the patient was initially diagnosed. Grey represents normal cells, tan represents cells exhibiting the *JAK2* mutation only, maroon represents cells exhibiting both *JAK2* and *BCR::ABL1*, and red represents cells in the blast phase carrying both *BCR::ABL1* and *JAK2*.

via FISH at the initial PMF diagnosis, it is plausible that a minute subclone, evading detection due to its size, was present and gradually underwent expansion. Roughly 20 months post-PMF diagnosis, the BCR::ABL1 clone achieved full expansion, becoming evident in 87% of cells according to FISH analysis. Concurrently, the *JAK2* V617F mutation still persisted with a notable VAF of 45.8%, indicating that the majority of cells were harboring both *JAK2* V617F and *BCR::ABL1*. Upon the onset of B-ALL diagnosis, *BCR::ABL1* fusion signals were solely detected in blasts, with mature granulocytes yielding negative results. Simultaneously, the VAF of *JAK2* V617F remained akin to the previous reading, measuring 41.2%. This phenomenon suggested that, at this stage, blasts carried the combined *JAK2* and *BCR::ABL1* mutations, while mature granulocytes retained only the *JAK2* mutation. Subsequent to a brief duration of chemotherapy, the *BCR::ABL1* clones were mostly eradicated, as evidenced by the absence of the *BCR::ABL1* fusion signal in blasts upon bone marrow biopsy following the therapy. Notably, the post-therapy biopsy demonstrated persistent PMF, underscoring the continued presence of the *JAK2* mutation.

In this case, blast transformation was associated with a complex karyotype showing the emergence of additional cytogenetic abnormalities other than previously detected t(9;22). Additionally, three ABL1 mutations were identified at the time of B-ALL diagnosis. These likely contributed to the disease's progression to the blast phase.

AUTHOR CONTRIBUTIONS

All authors have read and approved the manuscript. Devin Wang, Wataru Kamata, Fengxi Ye, and M. James You wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

FUNDING INFORMATION

None.

DATA AVAILABILITY STATEMENT

Not applicable as no datasets were generated in this study.

ETHICS STATEMENT

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

PATIENT CONSENT STATEMENT

The authors have confirmed patient consent statement is not needed for this submission.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

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