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Acute lymphoblastic leukemia arising in post-polycythemic myelofibrosis: a rare entity

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A clonal myeloproliferative neoplasm (MPN) of the hematopoietic stem cell, polycythemia vera (PV) involves increased red blood cell production, elevated red blood cell mass and variable increases in white blood cells and platelets [1]. PV can progress to myelofibrosis (MF), termed post-polycythemic MF (post-PV MF), which is characterized by ineffective bone marrow hematopoiesis, medullary fibrosis, extramedullary hematopoiesis and hypersplenism [1]. In advanced cases, patients experience bone pains, night sweats, weight loss, decreased quality of life and worsening performance status.

MPNs are genetically diverse diseases in which acquired mutations target the hematopoietic stem cell, dysregulating kinase signaling and cytokine expression, leading to clonal hematopoiesis. Further acquisition of secondary molecular/genetic aberrations is thought to lead to disease progression and, in some cases, transformation to acute leukemia [2–4]. Herein, we illustrate the “multiclonality” of this evolving process by describing a rare case of *JAK2*-exon 12 mutated post-PV MF that terminated in B-acute lymphoblastic leukemia (B-ALL). This process was accompanied by the emergence of an *NRAS* mutated clone and temporary disappearance of the *JAK2*-exon 12 mutated clone.

In January 2007, a 54-year-old male presented to his physician complaining of insomnia. Hypothyroidism, hypertension and elevated red blood cell count ($8.28 \times 10^9/L$), and hemoglobin (18.9 g/dL) were noted. Causes of secondary erythrocytosis were excluded and phlebotomy was initiated. After referral to a hematologist in August 2007, a bone marrow biopsy revealed a chronic MPN, with marked erythroid hyperplasia, increased immature erythroid precursors, increased atypical megakaryocytes and fibrosis, MF-2 (scale: 0–3). Peripheral blood showed: white blood cell count (WBC) $11.6 \times 10^9/L$, hemoglobin 16.1 g/dL and platelets $71 \times 10^9/L$. The bone marrow findings and history of erythrocytosis

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requiring phlebotomy suggested PV with progression to the more advanced phase, post-PV MF.

In November 2007, after five therapeutic phlebotomy treatments, he was referred to our hospital with a hemoglobin of 12.7 g/dL and splenomegaly 5 cm below the costal margin (CM). A repeat bone marrow biopsy confirmed the original diagnosis of post-PV MF. Findings included hypercellular bone marrow [Figure 1(A)] with increased fibrosis, MF-2 (scale: 0–3) [Figure 1(B)]. Iron staining was negative for ringed sideroblasts and also showed decreased iron stores.

Cytogenetic testing demonstrated a normal diploid male karyotype. Molecular studies were negative for the *JAK2V617F* mutation, but further *JAK2* analysis identified a deletion in exon 12 (codons 537–539). Fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR) studies were negative for the t(9;22) and *BCR-ABL1* transcript, respectively. Other laboratory studies revealed: low erythropoietin (4.5 mIU/mL), low serum iron (16 µg/dL), low ferritin (14 ng/mL), elevated uric acid (9.6 mg/dL), low platelets ($68 \times 10^9/L$), elevated lactate dehydrogenase (LDH) (1411 IU/L) and slight leukocytosis (WBC $12.3 \times 10^9/L$).

Over the next 3 years he remained asymptomatic despite slowly progressive splenomegaly and thrombo-cytopenia, with his spleen enlarging to 18 cm below the CM and with worsening leukocytosis (WBC $44 \times 10^9/L$). He declined investigational therapies, and over the next 8 months he developed abdominal discomfort, early satiety and fatigue. The spleen reached 28 cm below the left CM and the liver 18 cm below the right CM, with the WBC rising to $67 \times 10^9/L$ with 3% blasts. Hydrea 1 g twice daily was prescribed.

A bone marrow aspirate and biopsy were performed, demonstrating 40% blasts, which were small in size with high nuclear to cytoplasmic ratio, fine chromatin, one or two small distinct nucleoli and small amounts of agranular cytoplasm. The blasts were negative for myeloperoxidase (MPO) by cytochemical stain [Figure 1(c)], and positive for terminal deoxynucleotidyl transferase (TdT) by immunohistochemical stain [Figure 1(D)]. Flow cytometry studies revealed a blast population positive for CD10, CD19, CD20, human leukocyte antigen (HLA)-DR and TdT, while negative for CD13, CD14, CD15, CD33, CD34, CD64 and MPO. The morphologic and immunophenotypic findings supported the diagnosis of B-ALL arising from a chronic MPN. Molecular studies performed on the bone marrow sample no longer demonstrated the *JAK2*-exon 12 deletion. Instead, a new mutation in codon 12 of *NRAS* (GGT to GAT) changing the encoding amino acid Gly to Asp was detected by pyrosequencing. Malignant cells were present in the cerebrospinal fluid (CSF) but there were no neurologic abnormalities.

Chemotherapy with hyperfractionated cyclophosphamide, vincristine, doxorubicin (hyper-CVAD) (including intrathecal cytarabine and methotrexate) and rituximab was initiated. On day 11, his symptoms improved, and his hepatosplenomegaly regressed significantly: his spleen decreased to 10 cm below the left CM, and the liver decreased to 5 cm below the right CM. On day 29, the bone marrow contained only 2% blasts, with persistent MPN: hypercellular bone marrow (70–80%), dilated sinuses, intrasinusoidal hematopoiesis and

osteosclerosis [Figure 1(E)]. The megakaryocytes included large forms with hyperchromatic and hyperlobated nuclei [Figure 1(F)]. The *NRAS* mutation persisted, and the *JAK2*-exon 12 mutation remained undetectable. Malignant cells were cleared from his CSF.

After cycle 2 of chemotherapy, persistent severe thrombocytopenia and splenomegaly necessitated splenectomy, revealing a large spleen (1480 g) [Figure 1(G)] with extramedullary hematopoiesis, but no leukemia [Figure 1(H)]. Molecular studies performed on formalin-fixed paraffin-embedded splenic tissue again detected the *NRAS* alteration [Figure 1(I)] and reappearance of the original *JAK2*-exon 12 deletion [Figure 1(J)]. Following recovery from splenectomy the patient underwent an allogeneic stem cell transplant from an HLA-identical sibling, and is currently recovering with full engraftment. Prior to stem cell transplant, repeat bone marrow evaluation and molecular studies showed persistent MPN with identical *NRAS* and *JAK2* abnormalities.

Post-PV MF preceding ALL has been reported only once before [5]. This patient was a 53-year-old woman with a 17-year history of PV, which ultimately progressed to MF 1 year before development of B-cell ALL. At diagnosis with ALL, she had a malignant pleural effusion and multiple lymphoblastic skin tumors. She began therapy with vincristine, methotrexate and prednisone, resulting in a rapid regression of the pleural effusion, hepatosplenomegaly and cutaneous tumors. She achieved a partial hematologic remission but died at home within weeks of initiating therapy [5].

The outcome of patients with ALL arising from other MPNs (primary myelofibrosis [PMF], PV and acute MF [AML-M7]) has been poor, with most patients dying within a few months; our patient was the first to undergo transplant in complete remission (CR) [5,6].

JAK2 mutations are found in up to 99% of patients with PV. Over 95% of these mutations involve codon 617 of *JAK2* tyrosine kinase [7]. This results in constitutive activation of the *JAK2* kinase domain and autonomous erythropoietin-independent erythropoiesis [7]. The *JAK2*-exon 12 deletion, while uncommon among patients with MPNs, is functionally similar to the *JAK2V617F* mutation. In a recent study of *JAK2* mutational analysis of patients with MPNs, for every 100 *JAK2V617F*-positive patients, only 2.3 had *JAK2*-exon 12 mutations [8]. Patients with *JAK2V617F*-negative PV possessing *JAK2*-exon 12 mutations have demonstrated isolated erythrocytosis, without concurrent leukocytosis or thrombocytosis [9], consistent with the findings in our patient, who presented with a normal WBC, significant erythrocytosis, a hemoglobin of 18.9 g/dL and thrombocytopenia, rather than thrombocytosis. *JAK2* mutations are known to occur in ALL, and have been identified in nearly 20% of patients with Down syndrome-associated ALL [10].

Ras, a guanosine triphosphate (GTP)-dependent protein in the inner surface of the cell membrane, has intrinsic GTPase activity resulting in signal transduction from growth receptors to downstream effectors. The Ras protein is encoded by the *RAS* gene family that includes *KRAS*, *HRAS* and *NRAS*. *RAS* mutations, generally involving codons 12, 13 and 61, have been described in myeloid malignancies, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and atypical *BCR-ABL* negative MPNs, particularly chronic myelomonocytic leukemia [7]. *NRAS* mutations have rarely been identified in

chronic MPNs and in post-MPN AML (7–13%) [11]. Their role in the progression of MPN to acute leukemia is unknown [11,12].

While *NRAS* mutations occur in 20–30% of AML, the estimated incidence in pediatric ALL is only approximately 10%, and in adult ALL is unknown. The prognostic significance of *NRAS* mutations in ALL is uncertain [13]. In our patient, persistence of mutated *NRAS* post-chemotherapy suggests that the mutation involved the MPN clone rather than the B-lymphoblasts. Whether it occurred in the original MPN clonal population or was acquired in the process of progression and transformation is not clear.

Development of ALL in post-PV MF, with temporary disappearance of the *JAK2*-exon 12 deletion and identification of the *NRAS* mutation, illustrates the multiclonal nature of MPNs [2–4]. In patients with MPNs, it has been demonstrated that diverse, genetically distinct clones are acquired independently, and not from a shared founder clone [3]. Likewise, in patients with essential thrombocythemia, at least two frequently occurring and independent *JAK2V617F* events have been described, suggesting that the events take place in a polyclonal setting [4]. This case report is indicative of the myeloid cell's ability to transdifferentiate and express proteins usually found in ALL cells [14].

While MDS and AML are known complications of PV and are reported to result in death in up to 20% of cases [1], post-PV MF transforming to ALL is extremely rare. This case exemplifies the plasticity and clonal diversity of MPNs, with the ability to cross lineages perhaps through the acquisition of new molecular events [14].

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Figure 1.

(A) Bone marrow biopsy revealed hypercellular bone marrow. (B) Increased bone marrow fibrosis, MF-2 (scale: 0–3) (C) The blasts were negative for myeloperoxidase (MPO) by cytochemical stain. (D) The blasts were positive for terminal deoxynucleotidyl transferase (TdT) by immunohistochemical stain. (E) On day 29, the bone marrow contained only 2% blasts, with persistent MPN: hypercellular bone marrow (70 – 80%), dilated sinuses, intrasinusoidal hematopoiesis and osteosclerosis. (F) The megakaryocytes included large forms with hyperchromatic and hyperlobated nuclei. (G) After cycle 2 of chemotherapy, splenectomy revealed a large spleen (1480 g) (H) Examination of the spleen revealed extramedullary hematopoiesis, but no acute leukemia. (I) Molecular studies performed on formalin-fixed paraffin embedded splenic tissue again detected the *NRAS* alteration. (J) Molecular studies performed on formalin-fixed paraffin embedded splenic tissue detected reappearance of the original *JAK2*-exon 12 deletion.