Pediatric erythroblastic transformation of JAK 2-mutated prefibrotic primary myelofibrosis with concurrent PHF6 mutations

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To the Editor: Primary myelofibrosis (PMF) is a rare condition in children. According to the 2016 World Health Organization (WHO), classification of myeloproliferative neoplasms (MPN), PMF is divided into prefibrotic PMF (pre-PMF) and overt fibrotic PMF[1]. Pre-PMF is the proliferation of predominantly abnormal megakaryocytes and minimal or no reticulin fibrosis. Therefore, the lack of fibrosis in the early phase of thrombocytosis can be misdiagnosed as essential thrombocythemia[2]. One significant complication of MPN is leukemic transformation (LT); however, only a few cases of PMF in children have been reported[3]. The clinical utility of the three driver mutations in *JAK2* , *CALR, and MPL* has been shown, especially when *JAK2* is central to the pathogenesis of the MPN phenotype[4]. Additional mutations in *ASXL1, SRSF2, IDH1/2, or EZH2* have been shown[5]. *PHF6*is an X-linked tumor suppressor gene with a somatic mutation that causes an aggressive type of myeloid neoplasm[6]. Here, we report a case of pediatric*JAK2* -mutated pre-PMF with concurrent *PHF6* mutations that transformed into AML within a year of diagnosis.

A 14-year-old boy with no medical history was admitted to our hospital with lumbago. Physical examination revealed splenomegaly (5 cm below the costal margins). A complete blood count showed a white blood cell (WBC) count of 5.8×10^9/L, neutrophil count of 31%, lymphocyte count of 36%, red blood cell count of 7.34×10^9/L, hemoglobin concentration of 140 g/L, and platelet count of 1010×10^9/L. The patient was dia-

gnosed with essential thrombocythemia based on bone marrow findings, which showed hypercellularity (80-100%), increasing with separated circled-multinucleated megakaryocytes, hyper-segmented-megakaryocytes, atypical megakaryocytes, and micromegakaryocytes (Figure 1A), and was treated with anagrelide. At this time, there were no blasts or reticulin fibers. The patient had no karyotypic abnormalities. After written informed consent was obtained, target capture-based next generation sequencing (NGS) was performed on bone marrow DNA for the following genes: *MPL, ASXL1, CBL, JAK3, EZH2, IDH1, IDH2, JAK1, PHF6, SF 3B1, TET2, TP53, U2AF1, JAK2, NRAS/KRAS* and *IKZF1* by previous described methods[7]. Among these mutations, *JAK2 V617F* with mutant allele percentage 4% and *PHF6 p.Q121X* mutation with 64% were identified. In addition, we generated an MPN gene panel (*JAK2 V617F, JAK2 exon12, MPL W515L, MPL W515K, CALR type1-5*) using DNA microarray methods (SRL International Inc. Japan) and only the *JAK2V617F* mutation was identified. As the platelet count decreased, his symptoms became well-controlled. However, teardrop-shaped red blood cells and myeloblasts were observed in the peripheral blood six months later, and we performed a bone marrow biopsy. Results indicated hypercellularity (80-100%) with moderate fibrosis (MF grade 1; Figure 1B). Therefore, ruxolitinib was administered for myelofibrosis. Five months later, he showed elevated lactate dehydrogenase (LDH) levels and thrombocytopenia. Bone marrow aspiration revealed increased cellularity with predominant erythropoiesis and 40% erythroblasts (Figure 1C). Flow cytometric analyses revealed 14% glycophorin A and 90% CD34 positive blast cells. No reticulin fibrosis progression was observed. The cytogenetic analysis revealed a normal karyotype. The patient was diagnosed with acute erythroleukemia secondary to PMF. He underwent HLA haploidentical peripheral blood stem cell transplantation(haplo-HCT) from his mother, using post-transplantation cyclophosphamide (PT-Cy) for graft-versus-host disease (GVHD) prophylaxis. The conditioning regimen consisted of total body irradiation (12 Gy delivered in six fractions from days -8 to -6), fludarabine (30mg/m^2 from days -5 to -2), and cytarabine (3,000 mg/m^2×2 from days -5 to -4). GVHD prophylaxis consisted of high-dose PT-Cy (50 mg/kg intravenously on days 3 and 4) in combination with tacrolimus and mycophenolate mofetil from day 5 onward. Infused donor cells were 5.4×10^6/kg CD34 cells and 4.0×10^8/kg CD3 positive T cells. Engraftment occurred on day 21, and complete chimerism was achieved on day 33. He had several transplantation-related complications, including grade II acute GVHD (gut), which was treated with prednisolone; BK virus-associated hemorrhagic cystitis; and bronchiolitis obliterans syndrome (Supplementary Figure). He has been in complete remission for 7 years after transplantation. Considering that the bone marrow features are characterized by increasing cellularity with atypical megakaryocytes at clinical onset, the patient should first be diagnosed with pre-PMF.

Our patient developed AML (FAB M6) 11 months after the diagnosis of pre-PMF. In terms of time to progression, median time (range) to progression was 11.8 years (7.9-15.7 years) in pre-PMF[8]. According to the Dynamic International Prognostic Scoring System (DIPSS) Plus score, our case was classified as low-risk. Candidate genes contributing to LT from MPN to AML have been identified, including *TP53, TET2, ASXL1, EZH2, IDH1/2, RUNX1, U2AF1, NRAS/KRAS* , and *SRSF2* [5]. The adverse impact of molecular characteristics on survival in pre-PMF and overt PMF has been reported as a high mutation risk in *EZH2, ASXL1, IDH1, IDH2* , and *SRSF2* [8]. In our case, no additional somatic alterations were detected; however, a *PHF6* mutation was identified. Somatic *PHF6* mutations have been found in 2–3% of AML[6, 9]. The percentage of blasts in the bone marrow tends to be higher in patients with myeloid malignancies harboring *PHF6* mutations[6]. AML with high *PHF6* expression levels than controls correlated with shorter overall survival[10]. Furthermore, increased *PHF6* levels may be associated with CD34 positivity[10]. In a case series of MPN with increased fibrosis and blast crisis, 22 patients with *PHF6* mutations in MPN were enriched[11]. Thus, *PHF* 6 mutations can contribute to myeloid leukemic transformation in *JAK2* -mutated pre-PMF.

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Conflict-of-interest

The authors declare that there is no conflict of interest.

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Figure legends

Figure 1A. Bone marrow aspiration at first visit(day-370).

Square (A) shows a separated circular multinucleated megakaryocyte, (B) a hypersegmented megakaryocyte, (C) an atypical megakaryocyte, and (D) a micromegakaryocyte.

Figure 1B. Bone marrow biopsy on day-170.

Silver impregnation shows moderate myelofibrosis. Arrows indicate reticulin fibers.

Figure 1C. Bone marrow smear (1000×, May-Grunwald-Giemsa stain). The smear shows abnormal megakaryoblasts with round or oval nuclei, loose chromatin, agranular cytoplasm with blebs.

Supplemental Figure 1. Clinical course of patients.

A bone marrow biopsy on day-170 shows myelofibrosis, and bone marrow aspiration on day-30 shows erythroleukemia. aGVHD, acute graft-versus host disease; PSL, prednisolone; TBI, total body irradiation; FLU, fludarabine; Ara-C, cytarabine; CY, cyclophosphamide; MMF, mycophenolate mofetil; Haplo-SCT, haploidentical stem cell transplantation; BMA, bone marrow aspiration; BMB, bone marrow biopsy; BM, bone marrow.