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Two Cases of Myeloproliferative Neoplasm with a Concurrent *JAK2*^{V617F} Mutation and *BCR/ABL* Translocation without Chronic Myelogenous Leukemia Phenotype Acquisition during Hydroxyurea Treatment

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Dear Editor

Myeloproliferative neoplasms (MPNs) are categorized on the basis of *BCR/ABL* translocation occurrence as either Philadelphia-positive CML or Philadelphia-negative MPNs (Ph$^-$MPNs). MPNs include polycythaemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF) [1]. The *JAK2*^{V617F} mutation, which is specific for Ph$^-$MPN, occurs in more than 95% PV cases and in approximately 50% ET and PMF cases [1]. The 2008 revision of the WHO classifies MPNs into 2 categories, *BCR/ABL* positive CML and Ph-MPNs, on the basis of the presence or absence of the Philadelphia chromosome [2].

Although the 2008 WHO classification does not include MPNs with more than 1 genetic aberration as a distinct disease entity, some cases with coexistence of *JAK2*^{V617F} mutation and *BCR/ABL* translocation have been recently reported and up to 28 cases have been previously reported [3-20]. Among these reports, the majority of the patients either had pre-existing *BCR/ABL*-positive CML and developed *JAK2*^{V617F} mutation while undergoing tyrosine kinase inhibitor treatment [4-7] or developed *BCR/ABL*-positive CML with a pre-existing *JAK2*^{V617F} mutation-positive MPN [8-12]. In contrast, a small number of patients showed simultaneous occurrence of both *JAK2*^{V617F} mutation and *BCR/ABL* translocation, with a CML phenotype in the bone marrow (BM) with development of symptoms or morphology associated with *JAK2*^{V617F} mutation, and with MPN only after imatinib treatment [3, 6, 13-15]. We report 2 MPN cases that were simultaneously positive for both *JAK2*^{V617F} mutation and *BCR/ABL* translocation and that did not acquire the CML phenotype during hydroxyurea (Korea United Pharm, Seoul, Korea) treatment.

Case 1 was a 36-yr-old man who was admitted in November 2009 with severe thrombocytosis. The patient's hemogram results at admission were as follows: white blood cells, 9.4×10^9/L, Hb, 13.8 g/dL, and platelets, 830×10^9/L. The peripheral blood smear (PBS) and BM biopsy revealed a marked increase in the platelet and the clustered megakaryocyte numbers (9.2/high power field) without evidence of dysplasia. Allele-specific PCR for *JAK2*^{V617F} mutation detection and reverse-transcriptase PCR (RT-PCR) for *BCR/ABL* fusion transcript detection using custom-designed primers was performed. The PCR and the RT-PCR analyses showed that the patient was positive for *JAK2*^{V617F} heterozygous mutation and the *BCR/ABL* fusion transcript (b3a2

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type), respectively.

Subsequently, the *JAK2*^{V617F} mutation allele burden was quantified by real-time quantitative PCR using JAK2 Muta*Quant*^{TM} (Ipsogen, Marseille, France). The *JAK2*^{V617F} mutation allele burden was calculated as the percentage of the V617F copy number to the sum of V617F and wild-type copy number. The *JAK2*^{V617F} mutation allele burden was quantified to be 27.91% (7,200/25,800) at diagnosis. The major *BCR/ABL* fusion transcripts quantitation was also performed by real-time PCR using the LightCycler t(9;22) Quantification kit (Roche Diagnostics, Mannheim, Germany) and the normalized copy number (NCN) was 0.02 at diagnosis. The patient's karyotype was determined to be 46,XY [20]; however, his interphase FISH analysis result was nuc ish(ABL1x3,BCRx2)(ABL1 con BCRx1)[61/200], representing cryptic *BCR/ABL* fusion on der(22)t(9;22) in 30.5% of the total cells.

Thus, on the basis of these findings, the patient was diagnosed to have ET with a major *BCR/ABL* fusion transcript. The patient was treated with hydroxyurea and the initial response during the first year of treatment was promising (*BCR/ABL* fusion transcript maintained in the range of 0.005 NCN to 0.01 NCN). However, despite continuing treatment, the number of *BCR/ABL* fusion transcripts increased to 5.0 NCN in the second year of follow-up, which indicated treatment failure. Interestingly, the patient did not show morphological evidence of CML during the follow-up period.

Case 2 was a 58-yr-old man diagnosed with leukocytosis and splenomegaly on admission. The patient's hemogram results at admission were as follows: white blood cells, 19.7×10^9/L, Hb, 13.0 g/dL, and platelets, 285×10^9/L. The PBS showed an occasional presence of tear-drop cells and immature granulocytes with blasts (Fig. 1A). The BM biopsy showed extensive myelofibrosis (grade 2-3) with a cellularity of 90% and an increased number of dysplastic megakaryocytes (Fig. 1B). The myelofibrosis was demonstrated by the reticulin silver stain (Fig. 1C). At diagnosis, the *JAK2*^{V617F} mutation analysis showed a heterozygous mutation (Fig. 1D) and the *JAK2*^{V617F} mutation allele burden was quantified to be 69.66% (2,640/3,790). The RT-PCR analysis revealed the presence of *BCR/ABL* fusion transcript (b3a2 type) (Fig. 1E). The patient's karyotype was determined to be 46,XY,

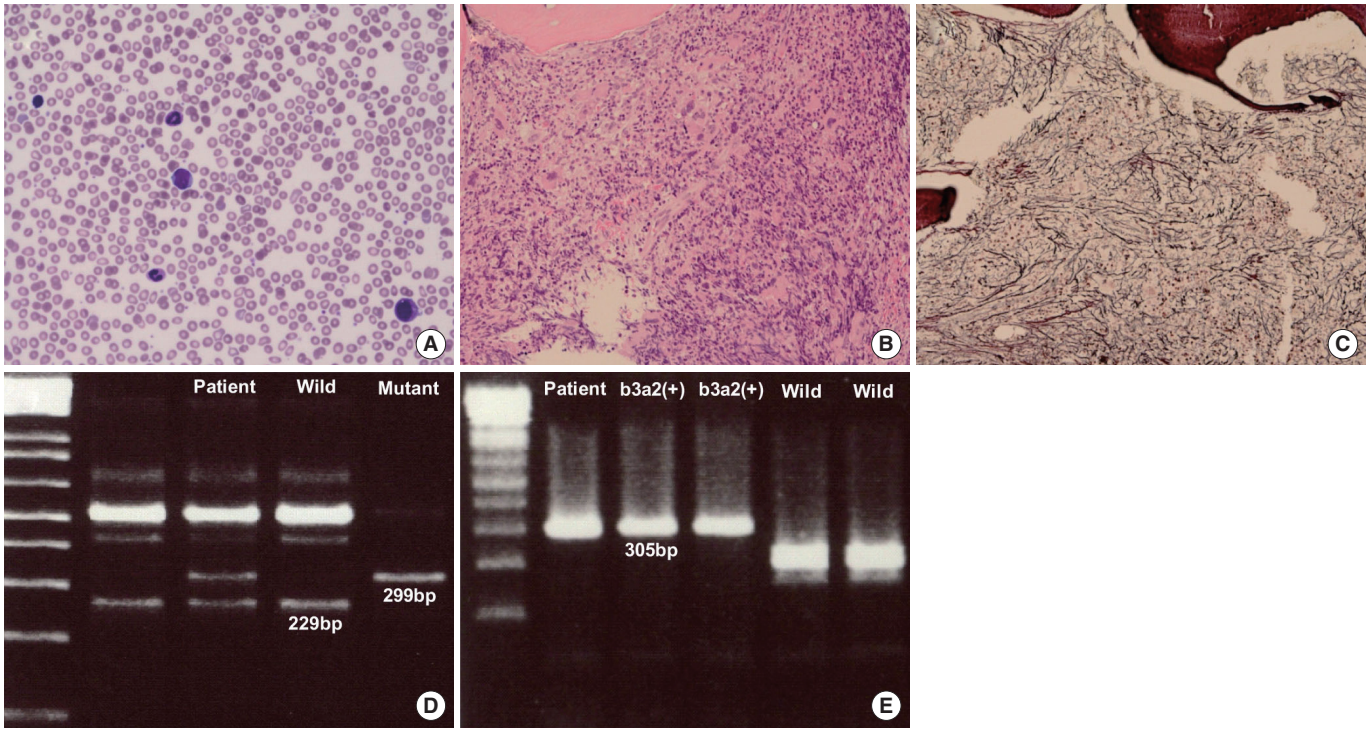

Fig. 1. The hematological and molecular characteristics of case 2. (A) A peripheral blood smear revealed tear-drop cells, immature granulocytes, and blasts (Wright stain, ×400). (B) The patient's bone marrow biopsy showed extensive myelofibrosis (H&E stain, ×100), demonstrated by reticulin silver stain (C, ×100) with cellularity of 90% and increased dysplastic megakaryocytes. (D) Mutational analysis of *JAK2*^{V617F} revealed bands that corresponded to both the mutant and wild-type *JAK2* (sized 299 bp and 229 bp, respectively). (E) Along with the myeloproliferative neoplasms (MPNs) diagnosis tests, reverse transcriptase (RT)-PCR analysis of *BCR/ABL* fusion transcripts was performed and a single 305-bp band, representing the major *BCR/ABL* translocation (b3a2 type), was obtained.

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t(9;22)(q34;q11.2)[4]/46,XY[16]. The quantification result for the major *BCR/ABL* fusion transcript in BM was found to be 1.0 NCN at diagnosis, which was 50-fold higher than that of case 1. The patient was treated with hydroxyurea for 6 months. However, the *BCR/ABL* fusion transcript levels remained at the levels at diagnosis (1.0-1.6 NCN). Similar to the findings for case 1, the morphological evidence of CML was not evident during hydroxyurea treatment. The drug treatment was changed to dasatinib and after 7 months of dasatinib treatment the patient did not show conversion, which was indicative of successful treatment.

Of the previously reported 28 cases with both *JAK2*^{V617F} mutation and *BCR/ABL* translocation, 15 patients had *BCR/ABL*-positive CML and acquired a *JAK2*^{V617F}-positive MPN phenotype after tyrosine kinase inhibitor treatment. Although 4 out of the 15 patients were diagnosed with CML and were shown to possess *JAK2*^{V617F} mutation, 3 of the 4 patients did not show a morphology associated with *JAK2*^{V617F} mutation-positive MPN until after treatment with imatinib. In contrast, 9 of the 28 patients showed *JAK2*^{V617F} mutation-positive MPN phenotype at initial diagnosis and developed *BCR/ABL*-positive CML during hydroxyurea treatment. Finally, at initial diagnosis, 4 patients showed mixed phenotype associated with both *BCR/ABL*-positive CML and *JAK2*^{V617F} mutation-positive MPN [3].

In recent studies, 2 hypotheses have been proposed to explain the coexistence of both *BCR/ABL* translocation and *JAK2*^{V617F} mutation in some patients. The first hypothesis which has been favored in the several literatures proposes that a single clone possesses one aberration and the patient's phenotype (e.g. CML feature) is dependent on the dominant clone (e.g. *BCR/ABL* translocation positive) which is determined by the selective pressure exerted by the specific treatment (e.g. hydoxyurea) prescribed for the other clone (e.g. *JAK2*^{V617F} mutation positive) [4-12]. The second hypothesis proposes that a single clone concurrently possesses both the *BCR/ABL* translocation and *JAK2*^{V617F} mutation [3, 13-15]. This hypothesis was supported by a recent study, which reported that the *BCR/ABL* translocation occurred in a pre-existing *JAK2*^{V617F} mutation-positive clone [18]. The data obtained in this study supports the second hypothesis as shown by the detection of concurrent *JAK2*^{V617F} mutation and *BCR/ABL* translocation at the initial diagnosis of MPN, and the lack of phenotype switch, especially to the CML phenotype, during hydroxyurea treatment. Hence, a comprehensive molecular genetic analysis is needed to elucidate the pathogenesis of these hematological chimeras.

In addition, the 2 patients showed different outcomes according to both the initial level of *BCR/ABL* fusion transcripts and the introduction of a tyrosine kinase inhibitor during the hydroxyurea treatment. The patient with low initial *BCR/ABL* fusion transcript levels experienced a relatively good initial response to hydroxyurea treatment, although the treatment failed 2 years later. In contrast, the patient with high *BCR/ABL* fusion transcript levels did not initially respond well to hydroxyurea treatment, but a dramatic response was achieved after a treatment change to dasatinib. On the basis of these findings, we speculate that treatment with a tyrosine kinase inhibitor can be effective and therefore recommend this approach in *JAK2*^{V617F}-positive MPN patients with a concurrent *BCR/ABL* translocation, particularly if the initial *BCR/ABL* fusion transcript level is high.

In conclusion, we report 2 cases of MPN with concurrent *JAK2*^{V617F} mutation and *BCR/ABL* translocation without CML phenotype acquisition during the hydroxyurea treatment. Treatment with tyrosine kinase inhibitors can be effective, particularly if the initial *BCR/ABL* fusion transcript level is high in these patients. Further molecular genetic analysis is needed to elucidate the pathogenesis of these hematological chimeras.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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