A Case Report of Chronic Myelogenous Leukemia With *JAK2*- and *BCR/ABL*-Positive Mutation

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Abstract

Myeloproliferative neoplasms (MPNs) include phenotypically diverse groups of clonal disorders, characterized by proliferation of 1 or more components of the myeloid lineage. Traditionally, chronic myelogenous leukemia (CML), essential thrombocytosis (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) were grouped together as MPNs.[1] Later, CML was found to be associated with a unique translocation between the long arms of chromosome 9 and 22 (*BCR/ABL* gene fusion), separating CML from other MPNs. The *JAK2* V617F (Janus–associated kinase) mutation and its association with ET, PV, and PMF was discovered in 2005. Traditionally, the coexistence of *JAK2* V617F and *BCR/ABL* was considered mutually exclusive.

Here we present a case of *JAK2 V*617F–positive PV, evolving to *BCR/ABL*–positive CML. Our case demonstrates there were 2 separate clones, harboring *JAK2 V617F* and *BCR/ABL* mutation. We have noticed an increase in the *JAK2* V617F mutational burden in patients who are administered tyrosine kinase inhibitor (TKI) therapy. Treatment with a TKI and a JAK inhibitor is the most reasonable approach in these patients. This case illustrates the importance of screening for *BCR/ABL* in PV or other MPNs when an atypical clinical course is encountered.

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Case Presentation

In 1999, a 68-year–old man presented with hematocrit of 50, and associated leukocytosis, erythrocytosis, thrombocytosis and splenomegaly (2-4 cm below the left costal margin). He was treated with hydroxyurea, aspirin, and intermittent phlebotomies (averaging 3-4 times per year). There were no major thromboembolic or bleeding events. A bone marrow (BM) biopsy performed in 2006 showed hypercellular marrow with trilineage hematopoiesis and megakaryocytic hyperplasia with focal clustering. There was no evidence of dysplastic changes. Focal mild increase in reticulin fibers was seen. This was similar to the BM biopsy performed at the time of his initial diagnosis in 1999. In 2010, he underwent a second BM biopsy (due to elevated white cell count from baseline 15-18 × 10^3/mcL to 24 × 10^3/mcL), which was no different from the earlier one, except for moderate grade 1-2 reticulin fibrosis. At this time, a full panel of molecular tests for myeloproliferative neoplasms (MPNs) was run. He was found to have *JAK2* V617F mutation with allele burden of 49%. Myeloproliferative leukemia mutation was negative. Cytogenetic analysis was positive for deletion 20q12 without any other chromosomal abnormalities.

In June 2012, there was a further increase in white blood cell count from baseline of 20 × 10^3/mcL – 30 × 10^3/mcL to 60 × 10^3/mcL – 80 × 10^3/mcL. At this time, the patient exhibited a progressive increase in splenomegaly (6-8 cm below the costal margin). There was concern for progression to secondary myelofibrosis or acute leukemia. At this point, a third BM biopsy was performed (13 years from the initial presentation and diagnosis of polycythemia vera [PV] and 2 years from his last BM biopsy). The BM was hypercellular with moderate reticulin fibrosis. There was no evidence of blast transformation. Cytogenetic analysis was positive for 2 abnormal clones; 14 cells were positive for t(9;22) (q34.q11.2), which was a new finding; and 6 cells exhibited deletion of the long arm of chromosome 20 (a previously known abnormality). There were no cells with dual abnormality. Fluorescent in situ hybridization (FISH) for *BCR/ABL1* rearrangement (break point: cluster Abelson) was positive (51.5% [103/200 nuclei]). Quantitative reverse transcription polymerase chain reaction for *BCR/ABL* transcript was 1.482% (e13a2). Thus, a case of chronic phase chronic myelogenous leukemia (CML) was diagnosed in this patient, who had a preexisting diagnosis of JAK2-positive PV for 12 years.

The patient was subsequently treated with dasatinib. Two weeks after that therapy, he was admitted to the hospital for shortness of breath and was found to have pleural effusions. Treatment was then switched to imatinib. He was able to achieve major molecular response with imatinib therapy; however, his splenomegaly persisted and he did not have a hematologic response.

During this time, the patient also developed constitutional

symptoms, such as fatigue and early satiety. Another BM biopsy was performed 6 months from the start of tyrosine kinase inhibitor (TKI) therapy. This was a dry tap, with no aspirate. The biopsy showed marked hypercellularity, extensive myelofibrosis, and no increase in blasts. Morphologic findings were consistent with post-PV myelofibrosis. Only 15 metaphases were able to be retrieved and all of them had deletion of 20q11.2. There were no metaphases with the Philadelphia chromosome.

Subsequently, the *JAK2* V617F allele burden increased from 49% to 80% (11 months since initiation of TKI therapy). He continued to be in complete molecular response, so the etiology for worsening counts and constitutional symptoms was thought to be from post–PV myelofibrosis. Ruxolitinib, a JAK inhibitor, was initiated, after which splenomegaly and constitutional symptoms improved. He was also started on erythropoietin therapy for anemia. After almost 21 months on TKI therapy and 10 months since starting ruxolitinib, we noted an increase in *BCR/ABL* levels. Mutational studies for the BCR/ABL kinase were negative. Mutational studies for *JAK2* were performed that revealed classic *JAK2* V617F with no new findings. Hence, a BM biopsy was performed (22 months from the diagnosis of CML and TKI initiation), which showed chronic phase CML with no blast phase. TKI therapy was switched to nilotinib. The patient again was able to achieve complete molecular response. He was treated with both the TKI and JAK inhibitor for over 3 years.

He was on erythropoietin (intermittently since 8 months from the diagnosis of CML) and subsequently started on danazol (3 years from the diagnosis of CML) for symptomatic anemia requiring blood transfusions. He eventually developed a significant decline in performance status and worsening B symptoms (3.5 years from the diagnosis of CML and 17 years from his initial diagnosis of PV). He also developed worsening anemia, thrombocytopenia, and splenomegaly. BM biopsy at this time (3.8 years from the diagnosis of CML) showed mild hypercellular BM with severe reticulin fibrosis (grade 3). Morphologic finding was indicative of development of secondary myelofibrosis with underlying MPNs. Bone marrow fluorescence in situ hybridization (FISH) was negative for *BCR/ABL*. Cytogenetic analysis was positive for persistent clone of cells with deletion of long arm of chromosome 20 (this cytogenetic abnormality was seen throughout the disease course since the PV diagnosis in 1999). Two cells showed additional abnormality for deletion of chromosome 7q. There were no cells with t(9; 22), indicating progressive disease is driven by JAK2-positive PV and post-PV myelofibrosis rather than CML.

During his long course, the patient developed pulmonary hypertension, which also progressed with time. Pulmonary hypertension was thought to be from long-standing myeloproliferative disorders and TKI use. He succumbed from the above complications at the age of 84.

Discussion

We report a case of *JAK2* V617F–positive PV for several years who developed chronic phase CML (*BCR/ABL* positive) while retaining a *JAK2* V617F–positive clone. Eventually, he also developed secondary myelofibrosis.

MPNs include a heterogeneous group of disorders, primarily CML, essential thrombocytosis (ET), PV, and primary myelofibrosis (PMF).[1] CML is characterized by Philadelphia chromosome translocation between the long arms of chromosome 9 and 22. Excluding CML, other MPN disorders are associated with mutations, such as *JAK2* V617F,[2] cMPL,[3] and calreticulin.[4] Of these, the *JAK2* V617F mutation is positive in more than 90% of patients with PV[5] and more than 50% of patients with ET or PMF.[5-8]

Traditionally, the existence of *BCR/ABL* and *JAK2* V617F

FIGURE 1. *BCR/ABL and JAK2* Mutation Over Time Period, Depicted in Relation to the TKI (Imatinib and Nilotinib) and Ruxolitinib Therapy.

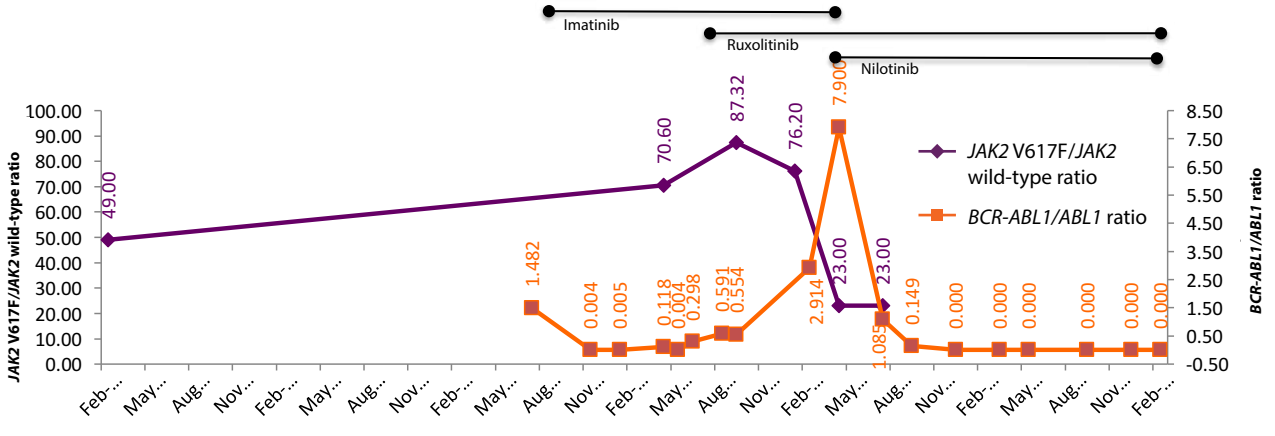
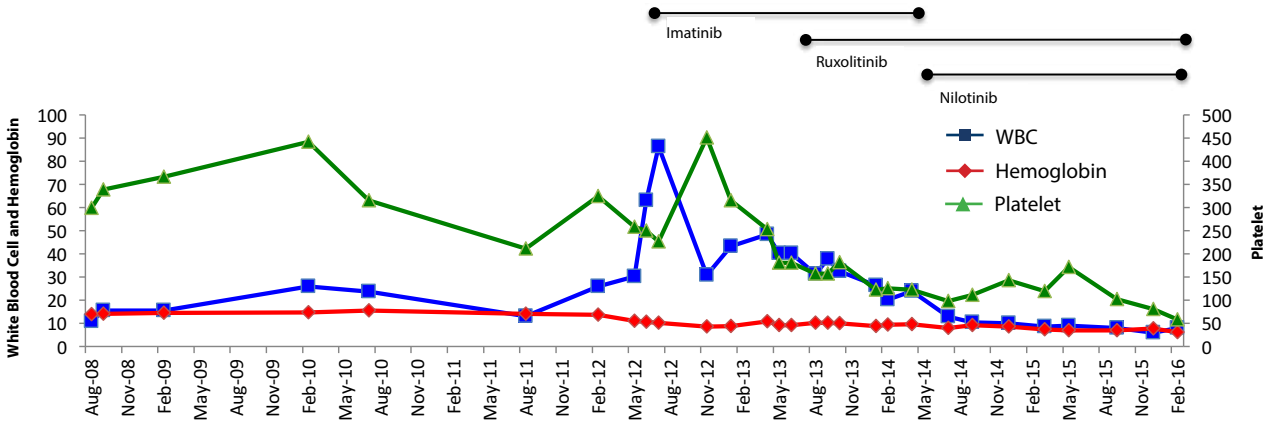


FIGURE 2. Trend of White Blood Cell Count, Hemoglobin, and Platelet Count Over Time in Correlation with the TKI (Imatinib and Nilotinib) and Ruxolitinib Therapy.



mutation were considered mutually exclusive.[9-11] However, there has been enough literature indicating that the coexistence of *JAK2* V617F and *BCR/ABL* is not that uncommon. It has been reported to occur concomitantly[7,12-16] or at transformation from ET,[17,18] PV,[8,19-21] or PMF[19] to CML. There have been reports of CML evolving to *JAK2*–positive MPNs.[12,22] Retrospectively, most of the later cases were found to have *JAK2* V617F at the time of CML diagnosis.[13] The emergence of *JAK2* V617F was also reported to occur after exposure to TKI therapy.[7,23,24] This is thought to result from unmasking of preexisting Philadelphia-negative MPNs due to CML treatment. Other possible explanations for this could be the fact that the Philadelphia chromosome's association with CML has been known since the 1960s, but *JAK2* V617F is a relatively new discovery. As a result, this could account for reporting bias due to lack of awareness.

Recently, the results of 2 studies have reported the incidence of *JAK2* V617F to range from 26% to 44%[16,25] in newly diagnosed CML cases, which is relatively higher than expected. It is not known which of these mutations occurs first. The majority of cases reported were JAK2–positive PV, ET, and PMF that later evolved to CML. Conchon et al and other studies reported acquisition of *BCR/ABL* to be a secondary event in a JAK2–positive clone.[26]

Whether coexistence of *JAK2* V617F in CML alters the natural course of the disease or not is not well known. The results of one study indicated that presence of the *JAK2* V617F mutation can predict early progression of CML.[16] Acquisition of *BCR/ABL* is also thought to induce more potent signaling leading to higher proliferation than *JAK2* V617F mutation.[19,21]

It is not well known if single clones of cells acquire dual abnormality versus separate subclones emerging with additional mutations. Few studies showed emergence of dual abnormality at the single cell level[7,21,23,27,28]; on the contrary, some studies showed emergence of abnormalities to be sequential in subclones.[29,30] A study by Yamada et al examined the CD34–positive cells at the time of CML emergence in a patient with JAK2–positive PMF and found that *BCR/ABL* translocation was acquired in the preexisting heterozygous JAK2–positive clones; interestingly, no colony contained *BCR/ABL* transcript in the absence of JAK2 mutation.[19,21] In our case, we have demonstrated that there were 2 separate clones: 1 harboring deletion 20q (which was seen throughout the disease course) and the other *BCR/ABL*. The 2 clones seem to have developed independent of each other since at no time could we identify evidence of a single cell with both the deletion 20 and the Philadelphia chromosome; it is possible that the patient had 2 independent MPNs that appeared sequentially. An alternative explanation could be that there was a common precursor and the 2 clones evolved separately.

Although TKI therapy in patients with *JAK2 V617F*–positive CML patients was successful in suppression of the *BCR/ABL* clone, it had no effect on the *JAK2* V617F clone.[29] The number of JAK2 clones increased while the patient was administered TKI therapy.[22,29-32] There was only 1 report in which TKI treatment of CML resulted in disappearance of the JAK2 clone after achieving partial cytogenetic response.[12]

We present a unique case in a patient who exhibited emergence of *BCR/ABL*–positive CML in preexisting *JAK2* V617F PV and who was treated with a TKI and a JAK inhibitor for over 3 years. To our knowledge, only 3 other cases[30,32] are reported in the literature where concomitant *JAK2* V617F– and *BCR/ABL*–positive CML was treated with a TKI and a JAK inhibitor.

Based on our case and review of literature, there is enough evidence supporting the coexistence of *JAK2* V617F and *BCR/ABL*; in fact, its incidence could be higher than reported. *BCR/ABL* testing should be conducted in cases like ours, when patients with longstanding JAK2–positive MPN develop a high white cell count or apparent disease progression, as CML may

TABLE. Depicting CBC (includes white blood cell, hemoglobin, and platelet count), *JAK2 V617F* Mutation Load, BCR/ABL Transcript Levels, Findings of the Bone Marrow Biopsy, Cytogenetics with Corresponding Treatments and Time Intervals.

	WBC (4.5-11.0 × 10^3/mcL)	Hemoglobin (14.0-18.0 g/dl)	Platelet (150-400 10^3 g/dl)	*JAK 2* V617F/ *JAK 2* wild-type ratio	*BCR-ABL* 1/*ABL* 1 ratio	Bone Marrow Biopsy	Cytogenetics	Therapy
2006						Hypercellular with trilineage hematopoiesis and megakaryocytic hyperplasia with focal clustering, no evidence of dysplatic changes. Focal mild increase in reticulum.		
Aug-08	11	14	299					Hydroxyurea and phlebotomy
Sep-08	15.7	14.2	339					
Feb-09	15.6	14.6	367					
Feb-10	26	14.8	442	49		Mild hypercellular marrow with trilineage hyperplasia marked megakaryocytosis with atypical megakaryocytes, absent iron, no blasts, 1.2 reticulin fibrosis.	Deletion 20	
Jul-10	23.8	15.6	316					
Aug-11	13.1	14.2	212					
Feb-12	26	13.7	325					
May-12	30.3	11.2	259					
Jun-12	63.1	10.8	250					
Jul-12	86.4	10.4	227		1.482	Hypercellular marrow with mild to moderate fibrosis, prominent megakaryopoiesis, increased storage iron, no blasts	14 metaphases with Philadelphia and 6 showed del 20	Dasatanib, then switched to imatanib shortly
Nov-12	30.9	8.7	451		0.004	Extensive fibrosis, dry tap	Only 15 metaphases with Philadelphia	Imatinib
Jan-13	43.4	8.8	316		0.005			Erythropoietin
Apr-13	48.5	10.9	253	70.6	0.118			
May-13	40.3	9.2	181		0.004			
Jun-13	40.2	9.2	181		0.298			Imatinib/ Ruxolitinib
Aug-13	31.3	10.2	158		0.591			

TABLE CONTINUED. Depicting CBC, *JAK2* V617F Mutation Load, *BCR/ABL* Transcript Levels, Findings of the Bone Marrow Biopsy, Cytogenetics with Corresponding Treatments and Time Intervals.

	WBC (4.5-11.0x 103/ul)	Hemoglobin (14.0-18.0g/dl)	Platelet (150-400 103 g/dl)	*JAK V617F/ JAK 2* wild type ratio	*BCR-ABL 1/ABL 1* ratio	Bone Marrow Biopsy	Cytogenetics	Therapy
Sep-13	37.8	10.2	158	87.32	0.554			
Oct-13	32.6	10.1	182				BCR/ABL kinase, no mutation detected	
Jan-14	26.4	8.8	123	76.2				
Feb-14	20.3	9.4	126		2.914	.	BCR/ABL kinase no mutation detected	
Apr-14	24	9.7	123	23	7.9	80%-90% cellularity, full maturation of neutrophils, no immature mononuclear cells, increased number with loose clustering of megakaryocytes, frequent abnormal forms, prominent marrow fibrosis	*JAK2* exons 12-15 mutation analysis- showed JAK2: NM_ 00492.3 (JAK2) :c.1849G, which tranlates to predicted protein change; p.va1617 phe (V617F)	Nilotinib, then continued with ruxolitinib
Jul-14	13	8	98	23	1.085			
Sep-14	10.6	8.7	143		0.149			
Dec-14	10	14.8	120	49	0			
Mar-15	8.6	7.4	120		0			
May-15	9	7	172		0			
Sep-15	8	7	102		0			Danazol
Dec-15	6	7.8	81		0			
Feb-16	8	6.3	59		0	Mild hypercellular, severe reticular fibrosis	2 cells with deletion of 20q, deletion 7, Philadelphia negative aPhiladelphia negative	

be concomitantly present. In our case, we demonstrated 2 separate clones independent of each other, which also are supported by the increase in the *JAK2* V617F allele burden and *BCR/ABL* levels after initiation of TKI and ruxolitinib therapy, respectively.

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