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Sequential Development of JAK2\(^{V617F}\) Mutation and BCR-ABL1 Fusion in Individual Patients With Myeloproliferative Neoplasms

A Linear Clonal Evolution or Parallel Clonal Competition?

Yue Zhao, MD, PhD; Deepthi Reddi, MD; Jenna McCracken, MD, PhD; Natasha Iranzad, MD; Cathrine Rehder, PhD; Jadee Neff, MD, PhD; Endi Wang, MD, PhD

**Context.**—Concomitant BCR-ABL1 and JAK2\(^{V617F}\) in myeloproliferative neoplasms (MPNs) is rare, and its pathogenesis and clinical significance are unclear.

**Objective.**—To investigate the clonal relationship between the 2 genomic alterations, as well as the clinicopathologic impact.

**Design.**—Retrospective analysis of MPNs with sequential development of BCR-ABL1 and JAK2\(^{V617F}\).

**Results.**—Of 6 cases, 5 had JAK2\(^{V617F}\)-positive MPN diagnosed before acquiring BCR-ABL1 years later, and 1 had BCR-ABL1\(^{+}\) chronic myeloid leukemia before JAK2\(^{V617F}\)-positive myelofibrosis completely replaced the BCR-ABL1\(^{+}\) clone 1 year after tyrosine kinase inhibitor therapy. Among the former group, treatment for the initial MPN involved hydroxyurea, ruxolitinib, and/or supportive care, and the latency to the development of JAK2\(^{V617F}\) ranged from 4 to 13 years (median of 9 years). Four cases showed retention of JAK2\(^{V617F}\), whereas BCR-ABL1 emerged as the major clone, including 2 that exhibited parallel increases in JAK2\(^{V617F}\) and BCR-ABL1 burdens, with both genomic markers exceeding 50%. Three patients received stem cell transplants and demonstrated sustained engraftment, with the genomic markers below detectable levels.

**Conclusions.**—Most MPNs with concomitant JAK2\(^{V617F}\) and BCR-ABL1 are actually composite MPNs with a “second hit” residing on a different clone. Rare cases demonstrate a subclone harboring a “double-hit” in a background of a JAK2\(^{V617F}\)-positive stem line clone. The probability of a “double-hit” with a BCR-ABL1 stem line clone is probably reduced by effective tyrosine kinase inhibitor treatment. The treatment often involves combined kinase inhibitors and/or hydroxyurea, but the outcome is unpredictable; hematopoietic stem cell transplantation may be the ultimate therapeutic option for this complicated disease.

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**B**CR-ABL1 fusion and JAK2\(^{V617F}\) mutation are 2 genomic aberrations defining different subtypes of myeloproliferative neoplasms (MPNs). The current World Health Organization classification scheme separates MPNs by the presence or absence of BCR-ABL1 fusion. Presence of the BCR-ABL1 gene fusion is diagnostic of chronic myeloid leukemia (CML), and patients with this underlying genomic aberration are eligible for treatment with tyrosine kinase inhibitors (TKIs) specifically targeted against the ABL kinase.\(^1\) Conversely, patients with neoplasms demonstrating similar morphology and clinical presentation but without laboratory evidence of BCR-ABL1 fusion are described as having “non-CML type” MPNs, and they do not benefit from treatment with ABL-targeted TKIs.\(^2,3\) Of the “non-CML type” MPNs, most harbor JAK2\(^{V617F}\) mutations, including >95% of polycythemia vera, 50% to 60% of essential thrombocytopenia, and 50% to 60% of primary myelofibrosis.\(^4,5\) A significant fraction of JAK2\(^{V617F}\)-negative MPNs are instead positive for CALR or MPL mutation.\(^5\) Although rare cases of myelodysplastic syndrome (MDS)\(^6\) and a few cases of MDS/MPN\(^7\) demonstrate JAK2\(^{V617F}\) mutation, its presence is usually associated with myeloproliferative features, such as thrombocytosis, highlighting a salient laboratory feature distinct from that seen in conventional MDS. Because the JAK2\(^{V617F}\) mutation is never found in hematopoietic elements of individuals without myeloid neoplasm, its detection helps define clonal myeloid disease in morphologically equivocal cases.\(^8\) BCR-ABL1 fusion and JAK2\(^{V617F}\) mutation have been considered mutually exclusive in individual cases; however, recent

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advances in genomic analysis have led to many case reports and several case series regarding MPN with concomitant BCR-ABL1 fusion and JAK2V617F mutation.9–17 The pathogenesis behind the concurrence is currently unknown, and the clonal relationship between the 2 genomic events remains to be investigated. Here, we report 6 cases with sequential development of JAK2V617F mutation and BCR-ABL1 fusion in individual patients with MPN.

MATERIALS AND METHODS

Case Selection
Six cases of MPN with concomitant JAK2V617F mutation and BCR-ABL1 fusion were identified from our bone marrow biopsy database. These included 4 cases from Duke University Medical Center (Durham, North Carolina) and 2 cases from the University of Washington (Seattle). The diagnosis of primary myeloproliferative neoplasm in each case was confirmed according to the 2017 update of WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.1–3 The clinical history and laboratory data for the patients were collected from corresponding clinical notes in our medical informatics systems.

Cytomorphologic And Histologic Evaluation
Peripheral blood smears were stained with Wright stain, bone marrow aspirate smears and biopsy touch imprints were stained with Wright–Giemsa stain, and bone marrow core biopsies and clot sections were stained with hematoxylin–eosin stain. The cases were reviewed independently by 2 hematopathologists (E.W. and D.R.). Bone marrow cellularity was evaluated on core biopsy, or on clot section in cases where the biopsy quality was suboptimal. The ratio of myeloid to erythroid elements was calculated from differential cell counts on bone marrow aspirate smear or touch imprint, or estimated on the histologic section of either bone marrow biopsy or clot, if the cytomorphologic sample was inadequate. The size of megakaryocytes, their nuclear lobation, and their distribution were evaluated on biopsy and/or clot sections.

Conventional Cytogenetic Studies
Cytogenetic analysis was performed on 2 to 4 mL of bone marrow aspirate from each case. Cultures from each specimen were initiated from the fresh, anticoagulated specimen in tissue culture medium. The cells were incubated for 24 and 48 hours without mitogen stimulation. Chromosome preparations, including harvesting and GTW banding, were made using standard methods. Cytogenetic abnormalities were classified according to the International System for Human Cytogenetic Nomenclature.

Fluorescence In Situ Hybridization for BCR-ABL1 Chromosomal Fusion
An interphase fluorescence in situ hybridization (FISH) was performed using Vyysis dual-color, dual-fusion BCR-ABL1 rearrangement probe. This probe is designed to detect the juxtaposition of the BCR locus and ABL1 gene sequences. The translocation involving BCR at 22q11.2 and ABL1 at 9q34, t(9;22)(q34;q11.2), is visible by the fusion of red color probe and green color probe. In total, 200 interphase nuclei were evaluated by 2 different technologists, and the percentage of positive cells was reported.

Quantitative Reverse Transcriptase–Polymerase Chain Reaction for BCR-ABL1 Fusion Transcripts
This assay used the QuantiteX minor and IS kits (Asuragen Inc, Austin, Texas) to quantitate BCR-ABL1 mRNA in peripheral blood or bone marrow using endogenous ABL1 transcripts as reference. The lower limit of detection for the minor kit was set at 0.0025% cells expressing BCR-ABL1 fusion transcripts, whereas the lower limit of detection for the IS kit was set at 0.002% cells expressing BCR-ABL1 fusion transcripts. Results for the p210 transcripts were reported on the International Scale.

JAK2V617F Mutation Analysis
JAK2V617F mutation was tested by 3 methods because of the chronological advance in detection techniques. These included qualitative allele-specific polymerase chain reaction (PCR) assay, semiquantitative PCR assay with allele-specific primers, and next-generation sequencing analysis of myeloid panel. All these assays used high-quality genomic DNA prepared from peripheral blood or bone marrow aspirate sample.

Qualitative Allele-Specific PCR Assay.—This assay used allele-specific primers targeting both wild-type JAK2 and mutant JAK2 alleles. After PCR amplification, fluorescently labeled products were separated by capillary electrophoresis. The presence of both PCR products, or the presence of only correctly sized mutant product, indicated that cells harboring JAK2V617F mutation were present in the sample. The assay had a detection limit of 0.1% in a background of wild-type genomes.

Semiquantitative PCR Assay.—This assay used a TaqMan real-time PCR-based technique to detect the V617F mutation (c.1849G>T) in the JAK2 gene. A single real-time PCR reaction was performed in each assay using an oligonucleotide primer pair that amplifies both the mutant and wild-type JAK2 alleles and allele-specific TaqMan probes that were fluorescently labeled with VIC/MMRA (wild-type JAK2) or FAM-TAMRA (V617F mutant JAK2). A threshold cycle value (Ct) was measured for each TaqMan probe. For each sample, a ΔCt value was calculated as the difference in Ct value between the JAK2 wild-type and JAK2V617F mutant probes. In addition, 1%, 50%, and 100% mutant controls, as well as wild-type control, were included in each assay. Results were interpreted based on the patient ΔCt value relative to the controls. This test was performed using an ABI Prism 7500 Sequence Detection System (Thermo Fisher Scientific Inc, Waltham, Massachusetts).

Next-Generation Sequencing Analysis of Myeloid Panel.—Specific regions of 75 target genes (including the hotspot mutation locus of the JAK2 gene) were sequenced using the Illumina NextSeq 500 (Illumina Inc, San Diego, CA). The resulting DNA sequence was assembled and aligned to the reference human genome version GRCh37/hg19. Sequence analysis was performed using the Archer Analysis bioinformatics pipeline. The lower limit of detection for this assay was 5%, meaning that a variant allele can be detected in a 95% wild-type allele background (or 10% neoplastic cell content). A depth of coverage of less than 100X was considered inadequate.

RESULTS
The clinical findings, pathologic features, and genomic evolution of MPN in 6 cases are summarized in the Table.

Clinical Findings
Of 6 patients, 4 were male and 2 were female. Age ranged from 46 to 77 years, with a median of 54 years at the time of initial presentation when diagnosis of primary MPN was made. Two of the patients had a prior history of regional radiotherapy for nonhematolymphoid neoplasms before their diagnosis of MPN (patients 4 and 6). In terms of the chronologic sequence of the 2 MPNs in individual patients, 5 patients (patients 1–5) received an initial diagnosis of JAK2V617F-positive MPN, and 1 patient (patient 6) received a diagnosis of BCR-ABL1–CML before developing JAK2V617F-positive primary myelofibrosis 1 year later. The 2 patients with polycythemia vera were initially treated with phlebotomy, followed by hydroxyurea, and both received additional treatment with ruxolitinib after developing splenomegaly and anemia. The other 3 patients with other JAK2V617F-positive MPN were initially treated with hydroxyurea (patients 3 and 4) or ruxolitinib (patient 5) before developing BCR-ABL1 fusion. In those 5 cases of JAK2V617F-positive MPN, the latency from diagnosis of JAK2V617F-positive MPN—Zhao et al
### Summary of Clinicopathologic Features of 6 Cases With Metachronous Development of JAK2V617F Mutation and BCR-ABL1 Fusion

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diabetes mellitus</th>
<th>First presentation</th>
<th>CBC</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
<th>Karyotype</th>
<th>FISH for BCR-ABL1</th>
<th>qRT-PCR for BCR-ABL1</th>
<th>Molecular test</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Disease course/interval</th>
<th>Second presentation</th>
<th>CBC</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
<th>Karyotype</th>
<th>FISH</th>
<th>qRT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>Male</td>
<td>NA</td>
<td>Erythrocytosis, thrombocytosis</td>
<td>NA</td>
<td>17/170 NA</td>
<td>Hyperplastic hematopoiesis with megakaryocytic hyperplasia of large lobate forms</td>
<td>46,XY[20]</td>
<td>Negative</td>
<td>Negative</td>
<td>JAK2V617F (&gt;80%)</td>
<td>46,XY</td>
<td>PV</td>
<td>Phlebotomy, hydroxyurea, ruxolitinib</td>
<td>Post-PV MF with gain of TP53 mutation in 6 y; BCR-ABL1 fusion in 9 y</td>
<td>32.7/85.95</td>
<td>Leukoctytosis, thrombocytopenia, splenomegaly</td>
<td>Hypercellular (95%), myeloid hyperplasia (WE = 5:1), megakaryocytic hyperplasia with large hypolobate/ hyperchromatic forms and myelofibrosis</td>
<td>46,XY; q19.22 (q34); q11.2</td>
<td>ND</td>
<td>BCR-ABL1 (48%)</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>Female</td>
<td>NA</td>
<td>Incidental findings of leukocytosis and erythrocytosis</td>
<td>17.2/150/1059</td>
<td>Leukoerythroblastosis</td>
<td>Slightly hypercellular bone marrow with megakaryocytic hyperplasia of large lobate forms</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td>JAK2V617F</td>
<td>JAK2V617F</td>
<td>ET</td>
<td>Phlebotomy, hydroxyurea, ruxolitinib</td>
<td>Initial improvement of CBC, then increasing splenomegaly; BCR-ABL1 fusion in 13 y</td>
<td>40.3/112/100</td>
<td>Leukoerythroblastosis, basophilia, eosinophilia</td>
<td>Myeloid hyperplasia, megakaryocytic hyperplasia of hyperchromatic forms, severe myelofibrosis</td>
<td>46,XY; del(21)(q22)(11.2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>Male</td>
<td>NA</td>
<td>Incidental finding of thrombocytosis</td>
<td>7.2/150/1059</td>
<td>Leukoerythroblastosis</td>
<td>Slightly hypercellular bone marrow with megakaryocytic hyperplasia of large lobate forms</td>
<td>45,XX, Robertsonian der(14:21)(q10;q10)</td>
<td>Negative</td>
<td>Negative</td>
<td>JAK2V617F</td>
<td>JAK2V617F</td>
<td>PMF, cellular phase</td>
<td>Hydroxyurea, dose adjusted per platelet count</td>
<td>54.3/144/576</td>
<td>Leukoerythroblastosis, basophilia, eosinophilia</td>
<td>CLL-like clonal B cells (9%), hypercellular, myeloid hyperplasia of hyperchromatic forms of severe myelofibrosis</td>
<td>46,XY, del(21)q10(11.2)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>Female</td>
<td>NA</td>
<td>Invasive ductal carcinoma of breast, status after lumpectomy/regional XRT</td>
<td>21.5/138/258</td>
<td>Marked left-shifted neutrophilia, basophilia</td>
<td>Hypercellular, myeloid hyperplasia (WE = 25:1), megakaryocytic hyperplasia with large lobate forms, mild to moderate myelofibrosis</td>
<td>46,XY,del(6)(q13;q21)</td>
<td>Negative</td>
<td>Negative</td>
<td>JAK2V617F</td>
<td>JAK2V617F</td>
<td>PMF, cellular phase</td>
<td>Hydroxyurea, later discontinued because of side effects</td>
<td>79.8/140/258</td>
<td>Leukoerythroblastosis, basophilia, eosinophilia</td>
<td>Hypercellular (100%), myeloid hyperplasia (WE = 30:1), megakaryocytic hyperplasia with small hypolobate forms, severe myelofibrosis, no increase in blasts</td>
<td>79.8/140/258</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>Male</td>
<td>NA</td>
<td>Dizziness</td>
<td>15-1-6/140/454</td>
<td>Marked left-shifted neutrophilia, basophilia</td>
<td>Hypercellular, megakaryocytic hyperplasia with small hypolobate forms, severe myelofibrosis</td>
<td>46,XY,del(6)(q13;q21)</td>
<td>Negative</td>
<td>Negative</td>
<td>JAK2V617F</td>
<td>JAK2V617F</td>
<td>PMF, cellular phase</td>
<td>Hydroxyurea, later discontinued because of side effects</td>
<td>79.8/140/258</td>
<td>Leukoerythroblastosis, basophilia, eosinophilia</td>
<td>Hypercellular (100%), myeloid hyperplasia (WE = 30:1), megakaryocytic hyperplasia with small hypolobate forms, severe myelofibrosis</td>
<td>79.8/140/258</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>Male</td>
<td>NA</td>
<td>LUQ fullness, splenomegaly by CT</td>
<td>46,XY</td>
<td>13/14/14</td>
<td>Hypercellular, megakaryocytic hyperplasia with small hypolobate forms, severe myelofibrosis with osteosclerosis</td>
<td>67,XY,del(21)(q22)(11.2)</td>
<td>Negative</td>
<td>Negative</td>
<td>JAK2V617F</td>
<td>JAK2V617F</td>
<td>CML, chronic phase</td>
<td>Ruxolitinib</td>
<td>46,XY</td>
<td>Leukoerythroblastosis, basophilia, eosinophilia</td>
<td>Megakaryocytic hyperplasia of hyperchromatic lobate forms, severe myelofibrosis with osteosclerosis</td>
<td>46,XY</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Notes:**
- **JAK2V617F:** V617F in JAK2
- **BCR-ABL1:** Fusion transcripts in BCR-ABL1
- **qRT-PCR:** Quantitative reverse transcription-polymerase chain reaction
- **ND:** Not done
- **NA:** Not applicable
- **Erythrocytosis:** Increase in red blood cells
- **Thrombocytosis:** Increase in platelets
- **Diabetes mellitus:** A chronic metabolic disorder characterized by hyperglycemia
- **Dizziness:** A feeling of disorientation or lightheadedness
- **LUQ fullness:** Left upper quadrant fullness
- **Splenomegaly:** Enlargement of the spleen
- **Leukoerythroblastosis:** Presence of both leukocytes and erythrocytes
- **Hypercellular:** Increased cellularity
- **Myelofibrosis:** Fibrosis of the bone marrow
- **Hypercellular (95%), myeloid hyperplasia:** 95% of the bone marrow is composed of myeloid cells
- **Hypercellular (100%), myeloid hyperplasia:** 100% of the bone marrow is composed of myeloid cells
- **Hypercellular (95%), myeloid hyperplasia with megakaryocytic hyperplasia:** 95% of the bone marrow is composed of myeloid cells, with a lesser degree of megakaryocytic hyperplasia
- **Hypercellular (100%), myeloid hyperplasia with megakaryocytic hyperplasia:** 100% of the bone marrow is composed of myeloid cells, with a significant degree of megakaryocytic hyperplasia
- **Hypercellular, myeloid hyperplasia:** Increased cellularity with a preponderance of myeloid cells
- **Hypercellular, megakaryocytic hyperplasia:** Increased cellularity with a preponderance of megakaryocytes
- **Hypercellular, myeloid hyperplasia with megakaryocytic hyperplasia:** Increased cellularity with a significant presence of both myeloid and megakaryocytic cells
- **Hypercellular, myeloid hyperplasia with megakaryocytic hyperplasia of hyperchromatic forms:** Increased cellularity with a preponderance of myeloid and megakaryocytic cells, with hyperchromatic forms
- **Splenomegaly, leukocytosis, anemia:** Enlarged spleen, increase in white blood cells, decrease in red blood cells
- **Left-shifted neutrophilia:** Increase in lower bands of neutrophils
- **Left-shifted neutrophilia, basophilia, eosinophilia:** Increase in lower bands of neutrophils, and basophils, eosinophils
- **Left-shifted neutrophilia, basophilia, eosinophilia:** Increase in lower bands of neutrophils, basophils, and eosinophils
- **Leukocytosis, anemia, thrombocytopenia:** Increase in white blood cells, decrease in red blood cells, and decrease in platelets
<table>
<thead>
<tr>
<th>Molecular test</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2&lt;sup&gt;V617F&lt;/sup&gt;</td>
<td>JAK2&lt;sup&gt;V617F&lt;/sup&gt; (84%), PT53 (40%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>JAK2&lt;sup&gt;V617F&lt;/sup&gt; (&lt;50%)&lt;sup&gt;b&lt;/sup&gt;, negative for CALR</td>
<td>JAK2&lt;sup&gt;V617F&lt;/sup&gt; (~18%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>JAK2&lt;sup&gt;V617F&lt;/sup&gt;</td>
<td>ND</td>
<td>JAK2&lt;sup&gt;V617F&lt;/sup&gt; (~35%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treatment and disease course</td>
<td>XRT spleen; TKI added; 46,XY(t(9;22)(q34;q11.2);[19]/46,XY,del(20))(q11.2q13.1)(1); 89.1% BCR-ABL1 transcripts; 93.5% JAK2&lt;sup&gt;V617F&lt;/sup&gt;/48.6% TP53 (4 mo after BCR-ABL1&lt;sup&gt;+&lt;/sup&gt;); HSCT</td>
<td>Nilotinib added</td>
<td>Imatinib, then ponatinib and hydroxyurea discontinued because of side effects, anagrelide added</td>
<td>Imatinib, switched to nilotinib, sunitinib, ponatinib, dasatinib, omacetaxine, and then ruxolitinib along with hydroxyurea, splenectomy, followed by HSCT</td>
<td>Ruxolitinib and nilotinib to achieve BCR-ABL1 transcripts of 1.169%; HSCT</td>
<td>Ruxolitinib, nilotinib, hydroxyurea, supportive care</td>
</tr>
<tr>
<td>Follow-up, mo</td>
<td>2 (4 after HSCT)</td>
<td>42</td>
<td>131 (49 after HSCT)</td>
<td>77 (17 after BCR-ABL1&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td>Alive with complete engraftment 2 mo after HSCT; normal female karyotype; negative FISH for BCR-ABL1; 0.0098% BCR-ABL1 by qRT-PCR</td>
<td>Died of sepsis, DIC, organ failure</td>
<td>Fluctuated BCR-ABL1 transcripts (&lt;1%~48%), with elevated WBC count and platelets during nadir of the transcripts; 17% CLL-like B cells; in very poor condition</td>
<td>Alive with normal CBCs; no detectable BCR-ABL1 transcripts or JAK2&lt;sup&gt;V617F&lt;/sup&gt; mutation; complete donor cell engraftment in both sorted T cells and myeloid components</td>
<td>Alive with no detectable BCR-ABL1 transcripts or JAK2&lt;sup&gt;V617F&lt;/sup&gt; mutation; complete donor cell engraftment in both sorted T cells and myeloid components</td>
<td>Persistent severe anemia, thrombocytopenia, and massive splenomegaly; low BCR-ABL1 transcripts below quantification; loss of follow-up for 4.5 y</td>
</tr>
</tbody>
</table>

Abbreviations: BUN, blood urea nitrogen; CBC, complete blood cell count (WBC, × 10<sup>9</sup>/L; hemoglobin, g/L; platelets, × 10<sup>9</sup>/L); CLL, chronic lymphocytic leukemia; CML CP, chronic myeloid leukemia, chronic phase; CT, computed tomography; DIC, disseminated intravascular coagulopathy; DLBCL, diffuse large B-cell lymphoma; ET, essential thrombocytopenia; FISH, fluorescence in situ hybridization; HSCT, hematopoietic stem cell transplant; LDH, lactate dehydrogenase; LUQ, left upper quadrant; M/E, myeloid to erythroid ratio; MF, myelofibrosis; NA, not available; ND, not done; PMF, primary myelofibrosis; PV, polycythemia vera; qRT-PCR, quantitative reverse transcription–polymerase chain reaction for BCR-ABL1 fusion transcripts; R-CHOP, rituximab, cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone; TKI, tyrosine kinase inhibitor; WBC, white blood cell; XRT, regional radiotherapy.

<sup>a</sup> The percentage of BCR-ABL1 fusion transcripts represents roughly the percentage of neoplastic cells, whereas the variant allele frequency detected by NGS or estimated by allele-specific PCR indicates the burden of neoplastic cells by duplicating the number (assuming heterozygosity of the mutations). Follow-up reflects the time interval between identification of the second genomic alteration and the outcome (in months).

<sup>b</sup> Semiquantitative real-time PCR assay; other JAK2<sup>V617F</sup> mutation analyses were performed with allele-specific PCR assay (detection limit, 0.1%).

<sup>c</sup> Next-generation sequencing analysis with the mutant allele frequency in parentheses.
to acquisition of BCR-ABL1 fusion ranged from 4 to 13 years, with a median interval of 9 years. Patient 6 received nilotinib for the treatment of CML before development of splenomegaly and subsequent diagnosis of JAK2\textsuperscript{V617F}-positive myelofibrosis.

**Pathologic Features and Genomic Analysis**

All 5 patients with an initial diagnosis of JAK2\textsuperscript{V617F}-positive MPN showed morphologic features of bone marrow biopsies consistent with the diagnosis of each patient given in the Table. Specifically, the biopsies in patients 1 and 2 exhibited hyperplastic hematopoiesis with proliferation of large lobate megakaryocytes in keeping with the diagnosis of polycythemia vera (Figure 1, A). Bone marrow biopsy of patients 3 showed slightly increased to normal cellularity with hyperplasia of large lobate megakaryocytes in line with the diagnosis of essential thrombocytemia (Figure 1, B). The biopsies in patients 4 and 5 demonstrated hypercellular bone marrow with pancytopenia including megakaryocytic hyperplasia (data not shown). Although significant fibrosis was not identified at the time, both patients developed severe myelofibrosis in subsequent biopsies, fulfilling the diagnosis of primary myelofibrosis. At the time of acquiring BCR-ABL1 fusion, all 5 patients with an initial diagnosis of JAK2\textsuperscript{V617F}-positive MPN demonstrated marked leukocytosis with left-shifted neutrophilia, and 4 of them exhibited leukaerythroblastosis. Patient 6 presented with left-shifted neutrophilia and basophilia at the initial diagnosis of CML and showed anemia and thrombocytopenia when a bone marrow biopsy identified JAK2\textsuperscript{V617F}-positive myelofibrosis. All 5 patients with secondary BCR-ABL1 fusion showed hypercellular bone marrow with myeloid hyperplasia and megakaryocytic hyperplasia (Figure 2). Of note, all 5 of these patients demonstrated hypolobate megakaryocytic nuclei, and all but 1 (patient 1) displayed relatively small megakaryocytes. The bone marrow biopsy in patient 1 was remarkable for large megakaryocytes with hypolobate nuclei, in contrast to the small megakaryocytes with hypolobate nuclei in the other 4. A total of 5 out of 6 patients demonstrated significant myelofibrosis, including patient 6, with severe myelofibrosis and osteosclerosis.

Of the 5 patients with initial JAK2\textsuperscript{V617F}-positive MPN, 2 patients (patients 1 and 4) had BCR-ABL1 fusion detected by chromosomal analysis, interphase fluorescense in situ hybridization (FISH) study, and quantitative reverse transcriptase–PCR, 1 patient (patient 3) by chromosomal analysis and reverse transcriptase–PCR, 1 patient (patient 5) by chromosomal analysis alone, and the remaining patient (patient 2) by quantitative reverse transcriptase–PCR alone. At the time of acquiring BCR-ABL1 fusion, 4 patients (patients 1–4) remained positive for JAK2\textsuperscript{V617F}, and the remaining patient (patient 5) did not have the mutation tested. Of the patients with retention of JAK2\textsuperscript{V617F}, 2 (patients 1 and 2) showed JAK2\textsuperscript{V617F} allele frequency higher than 50%, 1 showed it lower than 20% (patient 3), and the remaining patient (patient 4) had JAK2\textsuperscript{V617F} tested by qualitative allele-specific PCR assay, thus providing no quantitative data for the mutant allele. Of note, patients 1 and 2 demonstrated high proportions of cells harboring BCR-ABL1 fusion while retaining high allele frequencies of JAK2\textsuperscript{V617F} mutation. Patient 6 initially received tyrosine kinase inhibitor (TKI) therapy, achieving a 4-log reduction of BCR-ABL1 transcripts in 6 months, but developed splenomegaly, anemia, and thrombocytopenia approximately 1 year after the diagnosis of CML. Bone marrow examination demonstrated myelofibrosis with evidence of acquired JAK2\textsuperscript{V617F} mutation but a complete loss of BCR-ABL1 by interphase FISH and quantitative reverse transcriptase–PCR testing.

**Treatment and Clinical Outcome**

With regard to subsequent treatment after acquiring additional genomic alterations, 4 patients (patients 3–6) received TKI plus JAK kinase inhibitors and/or hydroxyurea. Patient 2 also received TKI but died of disease complications 4 months after acquiring BCR-ABL1 fusion. Patient 1 was treated with radiotherapy targeting his enlarged spleen, but his peripheral blood smear and bone marrow biopsy remained similar in morphologic features 4 months later, with parallel increases in the quantities of BCR-ABL1 and JAK2\textsuperscript{V617F} allele burdens (Table, treatment and disease course). In patient 3, the patient was treated with combined TKI and hydroxyurea, and his complete blood cell counts stayed within a relatively normal range, but the level of...
BCR-ABL1 transcripts fluctuated between <1% and 48% with periodic dose adjustment of TKI therapy. Patient 6 was treated with ruxolitinib, hydroxyurea, TKI, and supportive therapy and remained in a poor hematologic condition 26 months after diagnosis of JAK2 V617F-positive MPN. In patients 1, 4, and 5, the patients eventually received hematopoietic stem cell transplants and were alive with complete donor cell engraftment and without detectable JAK2 V617F or quantifiable BCR-ABL1 4, 49, and 17 months after transplantation, respectively.

**DISCUSSION**

Both JAK2 V617F mutation and BCR-ABL1 fusion are drivers leading to MPN, with a subtle difference in morphology and clinical manifestation between the 2 genomic alterations. The 2 genomic events were previously thought to be mutually exclusive, with only rare cases of both events concomitant in individual patients. Recently, Soderquist et al\(^{16}\) reported 11 cases of MPN with concurrent JAK2 V617F mutation and BCR-ABL1 fusion, estimating a prevalence rate of 0.4% among all MPNs tested for both genomic alterations. Through analysis of longitudinal samples in each individual case, they concluded that most instances of concomitant JAK2 V617F mutation and BCR-ABL1 fusion were actually composites of CML and BCR-ABL1- MPN, and in some cases the second MPN was initially cryptic and became unmasked only after TKI treatment. This phenomenon of biclonal myeloproliferative processes (Figure 3, combination of routes 1 and 2) has been described in many case reports,\(^{9,10,12–15,17}\) and it seems to explain the pathologic evolution in patient 6 and possibly in patients 3 to 5 of our series. In patient 6, the BCR-ABL1- MPN emerged only after CML declined to an undetectable level status after nilotinib therapy, reflecting an impact of clonal competition between the 2 MPNs. Although there was no sample intermediate between CML and BCR-ABL1- MPN to be tested for JAK2 V617F, which would thus demonstrate transitional change, the short interval between the 2 MPNs suggests the possibility of their composite during the transition or even before the initial diagnosis. A composite of the 2 neoplastic components is suggested by the cytogenetic study and molecular data for BCR-ABL1 and JAK2 V617F in patient 3. When BCR-ABL1- CML is present in conjunction with JAK2 V617F-positive MPN, it seems to play a dominant role compared with the JAK2 V617F clone, quickly replacing the latter before TKI treatment,\(^{15,16}\) as seen in patients 4 and 5 in our series. Many times, effective treatment with TKI reverses the relative prevalence of the 2 clones.\(^{12–16}\)

Conversely, patients 1 and 2 in our series demonstrated a high allele frequency of JAK2 V617F when BCR-ABL1 fusion

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**Figure 2.** Effect of secondary BCR-ABL1 fusion on the bone marrow morphology. A, Patient 1. Note the hypercellular bone marrow with increased large hypolobate megakaryocytes. B, Patient 3. C, Patient 4. D, Patient 5. Note the hypercellular bone marrow with increased small hypolobate megakaryocytes in images B through D (hematoxylin-eosin, original magnification \(\times200\)).
emerged with a high fraction of the fusion gene and/or fusion transcripts, suggesting a probable linear clonal evolution by acquiring this second genomic aberration on the existing JAK2V617F-positive clone (Figure 3, combination of routes 1 and 3). This is especially evident in patient 1, in whom chromosomal analysis revealed 2 separate clones, one with isolated t(9;22) and the other with isolated 20q deletion. It is possible that both clones harbored JAK2V617F mutation as well, given its high allele frequency (84%). With time, the fraction of Philadelphia chromosome and BCR-ABL1 fusion transcripts increased, whereas JAK2V617F remained high in allele burden, suggesting an expansion of JAK2V617F/BCR-ABL1 double-positive subclone (“double-hit” clone; route 3 in Figure 3). A JAK2V617F allele frequency approaching 100% may be explained by homozygous mutation or loss of heterozygosity of the JAK2 locus in this case. Of note, the previous investigations relied on the dynamic change of allele burdens between JAK2V617F and BCR-ABL1 in response to TKI to determine the clonal composition. This analysis may overlook or underestimate the possibility of clonal evolution to a “double-hit” MPN because a case with a “double-hit” subclone might respond to TKI treatment with a decline in BCR-ABL1 burden but persistence of JAK2V617F, resembling that of a composite MPN.3–17 On the other hand, a JAK2V617F-positive subclone evolved from BCR-ABL1+ CML (Figure 3, composition of routes 2 and 4), a reverse sequence of “double-hit,” has not yet been well described either at the clinical or genomic level, even though it is theoretically feasible.14 This may be explained by the sustained minimal level of the BCR-ABL1 clone in many patients due to effective TKI treatment, thus lowering the chance for a “second hit,” when CML occurs before the JAK2V617F mutation. Given the potential clinical application, we advocate the genomic analysis of chronicologic bone marrow biopsies to provide a mutational landscape and thus determine the genealogic tree in leukemia evolution.

The latency from JAK2V617F-positive MPN to BCR-ABL1 acquisition took 4 to 13 years with a median of 9 years in our series, which is similar to the median interval reported by Soderquist et al16 and others.14 In addition, this latency is reminiscent of the interval between MPN and its transformation to acute-phase or myelodysplastic syndrome.15,16 Of note, 4 of the 5 patients in our series were treated with hydroxyurea for their initial MPN. Although there is believed to be a low risk of mutagenesis with therapeutic doses of hydroxyurea,21 the rarity of secondary BCR-ABL1 in patients with primary MPN suggests acquisition of this fusion event via a natural course rather than an iatrogenic cause. Interestingly, the clinical presentation and morphology seem to correlate with clonal composition, with onset of leukocytosis and small hypolobate megakaryocytes observed when acquiring BCR-ABL1 fusion, and thrombocytosis and large lobate megakaryocytes seen after acquisition of JAK2V617F.16

Because of the rarity of the cases, standardized treatment for MPN with concurrent BCR-ABL1 fusion and JAK2V617F mutation has not been established yet. In most clinical scenarios, a combination of kinase inhibitors to target the 2 driver kinases or TKI combined with hydroxyurea has been applied, and it has been adjusted by focusing on the dominant clone when the clonal composition shifts along with the treatment.15 Although successful anecdotes have been reported, the overall clinical outcome appears to be poor, with approximately half of the patients dying of disease progression.16 It has been described that MPNs often demonstrate disease progression, such as myelofibrosis, when additional genomic aberration is evident, which may contribute to the poor clinical outcome. Furthermore, comorbidity of composite neoplasms or concurrent genomic alterations complicate the selection of therapeutic targets and prediction of pharmacologic toxicity. In our series, all the patients were treated with a combination of kinase inhibitors, and 3 patients received additional hydroxyurea after the second genomic alteration was identified. One patient (patient 2) died of disease complications soon after the second diagnosis, and 2 other patients (patients 3 and 6) were in very poor condition with unstable hematologic status despite periodic adjustment of their medications, reflecting a difficulty in controlling the disease and a reduced tolerance to pharmacologic side effects. Interestingly, 3 of our patients (patients 1, 4, and 5) eventually received allogeneic hematopoietic stem cell transplantation, and all demonstrated a sustained remission with complete donor cell engraftment with undetectable or unquantifiable (patient 1) genomic markers, suggesting an ultimate therapeutic approach at the present time. Hopefully, along with a better understanding of the molecular mechanism of this particular clinicopathologic phenomenon, novel therapeutic targets and treatment strategy may be introduced to provide optimal care for this particular patient population.

References


Figure 3. Graphic illustration of clonal evolution of dual JAK2V617F-positive and BCR-ABL1 myeloproliferative neoplasms in individual patients. Solid lines represent stem line genomic alterations that drive the primary neoplastic proliferation, and dashed lines indicate sideline genomic alterations that result in “double-hit” subclones. The impact of genomic events on morphology is reflected by the morphologic features of megakaryocytes, which are represented by large cells with dark nuclei, pink cytoplasm, and irregular unframed cytoplasmic borders, indicating those with large lobate nuclei (route 1), those with hypolobate nuclei (route 2), and those with the mixed features (routes 3 and 4).


