JAK2 Mutations and Myeloproliferative Neoplasms

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Introduction

“Myeloproliferative disorders (MPDs)” were first defined by William Dameshek in 1951: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).\(^1\) The upcoming revised World Health Organization (WHO) classification system for hematopoietic tumors included these four classic MPDs in a broader category of “myeloproliferative neoplasms (MPNs)” that includes, in addition, other “non-classic” MPNs: chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), hypereosinophilic syndrome (HES), systemic mastocytosis (SM), and “MPNs, unclassifiable”. Both ‘classic’ and ‘non-classic’ MPNs as clonal stem cell disorders.\(^2\) The disease causing somatic mutations in these disorders have been identified in some but not others: \(BCR-ABL\) in CML, \(FIP1L1-PDGFR\) and chromosomal translocations involving \(PDGFRB\) or \(FGFR1\) in molecularly-defined myeloid malignancies associated with eosinophilia.\(^2\) In addition, other mutations of potential pathogenetic relevance are in the process of being defined: SM-associated \(KIT\) mutations, \(JAK2V617F\) in PV, ET, and PMF; \(JAK2\) exon 12 mutations in PV; and \(MPLW515L/K\) in PMF and ET.\(^2\)

\textbf{\(JAK2V617F\) and other novel mutations in the classic MPNs}

The association of \(JAK2V617F\) with MPNs, including PV, ET, and PMF, was first reported in 2005.\(^3\)\(^-\)\(^6\) Since then, the mutation has also been described, at a lower frequency, in a spectrum of other myeloid disorders including non-classic MPNs and other chronic myeloid neoplasms including myelodysplastic syndrome (MDS).\(^7\)\(^,\)\(^8\) \(JAK2V617F\) is not found in lymphoid disorders,\(^9\)\(^-\)\(^12\) solid tumor\(^13\)\(^-\)\(^15\) or secondary myeloproliferation.\(^16\)\(^,\)\(^17\) \(JAK2V617F\) is a G to T exon 14 somatic mutation resulting in the
substitution of valine to phenylalanine at codon 617. In general, a high JAK2V617F allele burden might be essential for acquisition of the PV phenotype in vivo; this is accomplished by mitotic recombination that leads to homozygosity for JAK2V617F.3-5

Unlike the case with JAK2V617F, the other two sets of mutations associated with MPNs are restricted to patients with PV (JAK2 exon 12 mutations)18,19 or PMF/ET (MPLW515L/K).20,21 JAK2 exon 12 mutations include both in-frame deletions and point mutations. MPLW515L represents a G to T transition at nucleotide 1544 resulting in a tryptophan to leucine substitution at codon 515 of the MPL receptor. Both exon 14 (i.e. JAK2V617F) and exon 12 JAK2 mutations induce cytokine-independent cell line proliferation and a PV-like disease in mice. In contrast, MPLW515L induces a PMF-like disease in mice. Regardless, both JAK2 and MPL mutations result in hyperactivation of JAK-STAT signaling and are therefore vulnerable to inhibition by a JAK2 antagonist.2

New diagnostic approaches in the classic MPNs

True polycythemia may represent either PV or non-clonal erythrocytosis (i.e. secondary polycythemia) that is often, but not always, mediated by erythropoietin (Epo). Apparent polycythemia (i.e. with normal red cell mass) may result from either a reduction in plasma volume (relative polycythemia) or an inaccurate perception of an elevated erythrocyte volume that results from not appreciating high normal values of hematocrit. The term “inapparent PV” is used to describe PV patients in whom increased erythrocyte volume is masked by “normal” hematocrit because of a concomitant increase in plasma volume.22 Therefore, in a suspected case of PV, one has to exclude both secondary and apparent polycythemia whereas the possibility of inapparent PV should be
entertained in the presence of PV-characteristic clinical features, regardless of the hematocrit level.

The recent discovery of the almost invariable association between PV and a JAK2 mutation (either JAK2V617F or a JAK2 exon 12 mutation)\textsuperscript{23} has led to a revised WHO diagnostic criteria for PV that incorporates JAK2 mutation screening (Table 1).\textsuperscript{24} In routine clinical practice, one should start with both JAK2V617F mutation screening and serum Epo measurement when PV is suspected (Figure 1).\textsuperscript{25,26} A positive mutation test is highly suggestive of the diagnosis, especially if accompanied by subnormal serum Epo level. Approximately 5\% of PV patients are negative for JAK2V617F.\textsuperscript{23} Therefore, in a JAK2V617F-negative patient with either a high clinical suspicion of PV or subnormal serum Epo level, the possibility of exon 12 JAK2 mutations should be entertained and pursued.

The 2001 WHO diagnostic criteria for ET and PMF have also been recently revised by the WHO (Tables 2 and 3).\textsuperscript{24} Unlike the case with PV, JAK2V617F mutation screening plays a minor role in the diagnosis of ET and PMF, which is primarily based on bone marrow histology;\textsuperscript{27} characteristic changes include hypercellularity, increased number of dysplastic megakaryocytes including cluster formation, and bone marrow stromal changes including myelofibrosis.\textsuperscript{28} In general, I also advise baseline bone marrow examination for all patients with PV although the procedure is not essential for making the diagnosis and its prognostic value has not been systematically studied.

**Prognosis and treatment of the classic MPNs in the JAK2V617F era**

In general, neither the presence of JAK2V617F nor its allele burden has been shown to influence survival or leukemic/fibrotic transformation in ET, PV, or PMF.\textsuperscript{29-37}
Similarly, the results of published studies regarding the relationship between thrombosis and \( \text{JAK2V617F} \) have not been consistent enough to warrant definitive conclusions in that regard. Instead, new information suggests a prognostic value for leukocytosis for overall survival in both ET and PV, for leukemic transformation in PV, and for thrombosis in both PV and ET.

In table 4, I have summarized current risk-adapted treatment strategies in ET, PV, and PMF. Phlebotomy is the only form of treatment modality that is believed to have improved survival in PV patients. In this regard, a recent study has challenged the need for aggressive phlebotomy in aspirin-treated patients with hematocrit that ranges between 40% and 55%. Nevertheless, for now, I recommend keeping the hematocrit level below 45%. In addition, all patients with PV should receive daily low-dose aspirin in the absence of contraindication for aspirin use. The additional benefit of cytoreductive therapy in PV, in terms of reducing thrombosis risk, was suggested by the early PVSG studies. However, the danger of long-term drug-associated complications has restricted their use in high-risk patients only (Table 4). At present, hydroxyurea is my drug of choice for use in high-risk patients with PV. The potential value of alfa interferon (\( \alpha \)-IFN) therapy requires validation in a controlled setting.

It is reasonable to expect near-normal survival in the majority of patients with ET, especially in the absence of leukocytosis, regardless of specific treatment. Furthermore, the relatively low incidence figures of thrombosis and hemorrhage as well as the occurrence of both short-term and long-term drug side effects are the basis for carefully selecting the patients with ET who require specific treatment (Table 4). In other words, at present, I use cytoreductive therapy in ET only in the presence of risk factors for
thrombosis including advanced age (≥ 60 years) or history of major thrombosis. In particular, I do not treat extreme thrombocytosis per se in the absence of aspirin-resistant symptoms. If cytoreductive therapy is indicated, then it is important to note that only hydroxyurea, as a treatment agent, has been shown in a prospective study to be associated with a reduced risk of thrombosis in ET. Hydroxyurea was also found to be superior to anagrelide in a head-to-head comparative study of high-risk patients with ET. The concern regarding hydroxyurea leukemogenicity in ET is currently unsubstantiated and the indiscriminate use of new drugs that are not tested in a controlled setting is unwarranted.

Among the BCR-ABL-negative classic MPNs, PMF has the worst prognosis with an approximate median survival of 5 years. However, several studies have identified both clinical and laboratory parameters that are used to identify good-risk as well as high-risk patient categories. The most important indicators of adverse prognosis are the presence of anemia (hemoglobin < 10 g/dL), advanced age (> 64 years), hypercatabolic symptoms (weight loss, profound fatigue, night sweats, low-grade fever), leukocytosis (>30,000/μL) or leukopenia (<4000/μL), circulating blasts (≥1%), high-risk cytogenetic abnormalities (+8, 12p-), peripheral blood monocyte count of ≥1,000/μL, and platelet count of < 100,000/μL. Table 4 outlines the Mayo prognostic scoring system for PMF and the corresponding treatment options for each risk category. The presence of del(5q) in intermediate- or high-risk patient with MF warrants a therapeutic trial with lenalidomide.
Table 1. Revised World Health Organization criteria for polycythemia vera (Tefferi et al. Blood 2007, by permission). Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria.**

**Major criteria**

1. Hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume*
2. Presence of JAK2V617F or other functionally similar mutation such as JAK2 exon 12 mutation

**Minor criteria**

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro

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*Hemoglobin or hematocrit > 99th percentile of method-specific reference range for age, sex, altitude of residence

or Hemoglobin > 17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from an individual’s baseline value that can not be attributed to correction of iron deficiency,

or Elevated red cell mass > 25% above mean normal predicted value
Table 2. Revised World Health Organization criteria for essential thrombocytemia 
(\textit{Tefferi et al. Blood 2007, by permission}).\textsuperscript{24} Diagnosis requires meeting all four criteria.

1. Sustained\textsuperscript{a} platelet count \(\geq 450 \times 10^9/L\)

2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes. No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis

3. Not meeting WHO criteria for polycythemia vera,\textsuperscript{b} primary myelofibrosis,\textsuperscript{c} chronic myelogenous leukemia,\textsuperscript{d} myelodysplastic syndrome,\textsuperscript{e} or other myeloid neoplasm

4. Demonstration of \textit{JAK2}\textsuperscript{V617F} or other clonal marker, or in the absence of a clonal marker, no evidence for reactive thrombocytosis\textsuperscript{f}

\textit{a. During the work-up period.}
\textit{b. Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels and red cell mass measurement is not required.}
\textit{c. Requires the absence of relevant reticulin fibrosis, collagen fibrosis, peripheral blood leukoerythroblastosis, or markedly hypercellular marrow for age accompanied by megakaryocyte morphology that is typical for primary myelofibrosis – small to large with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei and dense clustering}
\textit{d. Requires the absence of BCR-ABL}
\textit{e. Requires absence of dyserythropoiesis and dysgranulopoiesis}
\textit{f. Causes of reactive thrombocytosis include iron deficiency, splenectomy, surgery, infection, inflammation, connective tissue disease, metastatic cancer, and lymphoproliferative disorders. However, the presence of a condition associated with reactive thrombocytosis does not exclude the possibility of essential thrombocytemia if the first three criteria are met.}
Table 3. Revised World Health Organization criteria for primary myelofibrosis.

Diagnosis requires meeting all three major criteria and two minor criteria. (Tefferi et al. Blood 2007, by permission).

Major criteria
1. Presence of megakaryocyte proliferation and atypia,\textsuperscript{a} usually accompanied by either reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (i.e. pre-fibrotic cellular-phase disease).
2. Not meeting WHO criteria for polycythemia vera,\textsuperscript{b} chronic myelogenous leukemia,\textsuperscript{c} myelodysplastic syndrome,\textsuperscript{d} or other myeloid neoplasm
3. Demonstration of JAK2V617F or other clonal marker (e.g. MPLW515L/K), or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases\textsuperscript{e}

Minor criteria
1. Leukoerythroblastosis\textsuperscript{f}
2. Increase in serum lactate dehydrogenase level\textsuperscript{f}
3. Anemia\textsuperscript{f}
4. Palpable splenomegaly\textsuperscript{f}

\textsuperscript{a} Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering

\textsuperscript{b} Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels. Red cell mass measurement is not required

\textsuperscript{c} Requires the absence of BCR-ABL

\textsuperscript{d} Requires absence of dyserythropoiesis and dysgranulopoiesis

\textsuperscript{e} Secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies. It should be noted that patients with conditions associated with reactive myelofibrosis are not immune to primary myelofibrosis and the diagnosis should be considered in such cases if other criteria are met

\textsuperscript{f} Degree of abnormality could be borderline or marked
Table 4. Current treatment in *BCR-ABL*-negative classic myeloproliferative neoplasms

<table>
<thead>
<tr>
<th>Risk Categories</th>
<th>Essential Thrombocythemia (ET)</th>
<th>Polycythemia Vera (PV)</th>
<th>Primary myelofibrosis (PMF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low-dose aspirin</td>
<td>Low-dose aspirin</td>
<td>Age &lt; 50 years</td>
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<tr>
<td></td>
<td></td>
<td>+ Phlebotomy</td>
<td>Observation or</td>
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<td></td>
<td></td>
<td></td>
<td>Experimental drug therapy</td>
</tr>
<tr>
<td>Low but with</td>
<td>Low-dose aspirin**</td>
<td>Low-dose aspirin</td>
<td>Age ≥50 years</td>
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<tr>
<td>extreme</td>
<td></td>
<td>+ Phlebotomy</td>
<td>Observation or</td>
</tr>
<tr>
<td>thrombocytosis*</td>
<td></td>
<td></td>
<td>Experimental drug therapy</td>
</tr>
<tr>
<td>for ET and PV</td>
<td></td>
<td></td>
<td>or</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Low-dose aspirin**</td>
<td>Low-dose aspirin</td>
<td>Experimental drug therapy</td>
</tr>
<tr>
<td>for PMF</td>
<td></td>
<td>+ Phlebotomy</td>
<td>or</td>
</tr>
<tr>
<td>High</td>
<td>Low-dose aspirin + Hydroxyurea</td>
<td>Low-dose aspirin</td>
<td>Experimental drug therapy</td>
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<td></td>
<td></td>
<td>+ Phlebotomy +</td>
<td>or</td>
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<td></td>
<td></td>
<td>Hydroxyurea</td>
<td>RIC*** transplant</td>
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</tbody>
</table>

*Extreme thrombocytosis is defined as a platelet count of 1000 x 10^9/L or more.

**Clinically significant acquired von Willebrand disease (ristocetin co-factor activity < 30%) should be excluded before the use of aspirin in patients with a platelet count of over 1000 X 10^9/L.

***RIC, reduced intensity conditioning

**Risk stratification for essential thrombocythemia and polycythemia vera:**
- **High risk:** Age ≥60 years or previous thrombosis
- **Low-risk:** Neither of the above

**Risk stratification of PMF according to the Mayo Prognostic Scoring System:**
(One point each for hemoglobin <10 g/dL, leukocyte count <4 or >30 x 10^9/L, platelet count <100 x 10^9/L, or monocyte count ≥ 1 x10^9/L)
- **Low-risk:** score 0
- **Intermediate-risk:** score 1
- **High-risk:** score ≥2
Figure 1. Routine clinical practice algorithm for suspected myeloproliferative neoplasm (MPN) including polycythemia vera (PV) and essential thrombocythemia (ET). By permission from the Tefferi A. The Cancer Journal, in press.

Peripheral blood mutation screening for *JAK2V617F*

- Positive
  - Highly suggestive of an underlying myeloproliferative neoplasm
    - Bone marrow examination advised
- Negative
  - Does not rule out an underlying myeloproliferative neoplasm
    - PV suspected
      - Serum erythropoietin level measurement
        - Below normal
          - Bone marrow examination and *JAK2* exon 12 mutation screening advised
        - Normal or increased
          - Likely to PV
    - Other MPN suspected
      - Bone marrow examination advised if clinical evaluation does not suggest secondary myeloproliferation
References