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Perspective

The puzzle of myeloproliferative neoplasms: novel disease-specific mutations and new proposals for diagnostic criteria

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Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are collectively called "Philadelphia-negative classical myeloproliferative neoplasms" (MPNs), and the discovery of the *JAK2*V617F mutation in 2004 led us to make new progress in the diagnostic approach and therapeutic strategy for MPNs. Thereafter, other clonal markers, such as mutations of the *MPL* or *CALR* genes, were discovered and listed as useful markers for the distinction of MPN from reactive myeloproliferation.

The identification of recurrent gain-of-function somatic mutations in the *JAK2* and *MPL* genes during the years 2005–2007 provided critical insights into non-*BCR/ABL1* MPNs that have advanced our understanding of the molecular pathophysiology of these diseases. The *JAK2* and *MPL* mutations were readily incorporated into the diagnostic criteria for PV, ET, and PMF in the 2008 World Health Organization (WHO) classification [1]. Thereafter, the growing application of high-throughput sequencing technologies to the identification of genetic alterations at the nucleotide level has revealed a long list of genes, other than *JAK2* and *MPL*, which are mutated in MPNs. It is expected that 2 of these genes, *CALR* and *CSF3R*, will be added to the upcoming WHO classification, based on their significant frequency of occurrence and genotype-phenotype correlation, particularly in terms of therapeutic and prognostic implications [2]. The *CALR* gene will appear in the diagnostic criteria for ET and PMF; *CALR* mutations are reported to be detected in ~70% of *JAK2*-nonmutated ET and ~85% of *JAK2*-nonmutated PMF. *CALR* encodes the protein calreticulin, which has multiple functions and plays roles in, for example, cell proliferation and apoptosis. *CALR* mutations occur exclusively in exon 9 (the last exon) and are most commonly small deletions or insertions, with or without substitutions. The two most common mutations that account for ~80% of all *CALR* mutations, c.1092_1143del (p.L367fs*46) and c.1154_1155insTTGTC (p.K385fs*47), have been designated as type 1 and 2, respectively. The mutations both result in a frameshift to an alternative reading frame and generate a novel amino acid sequence at the C-terminus of the protein. Clinically, compared to patients with *JAK2* mutations, patients with *CALR* mutations have a higher platelet count, lower Hb and leukocyte levels, a lower risk of thrombosis, and a more indolent disease course [3]. Interestingly, mutation type was found to be associated with disease subtype (predilection for type 1 mutations in PMF) and with the clinical course of the disease (shorter survival with type 2 mutations in PMF) [4]. Technically, PCR and amplicon-sizing analyses can detect *CALR* mutations with high sensitivity and provide quantitative information, and direct sequencing analysis can then fully characterize the mutations. Of note, the 3 major driver mutations in non-*BCR/ABL1* MPNs, *JAK2*, *MPL*, and *CALR*, occur in an almost mutually

Table 1. Major driver gene mutations in *BCR/ABL1*-negative myeloproliferative neoplasms.

Gene[a]	Mutation frequency by disease subtype	Mutation hotspot	Mutation type	Molecular methods of mutation detection	Comment	WHO diagnostic criteria
JAK2 V617F	~95% in PV ~60% in ET or PMF	V617F	Gain-of-function missense	qPCR Direct sequencing	Significance of mutant allele burden	Included in 2008
JAK2 exon 12	~5% in PV	Exon 12	Gain-of-function missense, small ins/del	Direct sequencing	-	Included in 2008
MPL	~5% in ET or PMF	M515L/K/A	Gain-of-function missense	Direct sequencing qPCR, AS-PCR	-	Included in 2008
CALR	~30% in ET or PMF	Exon 9	Gain-of-function small ins/del	PCR amplicon-sizing analysis Direct sequencing	More favorable prognosis than *JAK2* mutation carriers	Pending
CSF3R	~90% in CNL[b]	-	Gain-of-function missense/nonsense	Direct sequencing		Pending

[a]*JAK2, MPL,* and *CALR* mutations typically occur in a mutually exclusive manner. [b]*CSFR* mutations also occur in ~45% of *BCR/ABL1*-negative atypical CML.
Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; CNL, chronic neutrophilic leukemia; ins/del, insertion/deletion; qPCR, quantitative real-time PCR; AS-PCR, allele-specific PCR.

exclusive manner in PV, ET, and PMF. In addition, a mutation of the *CSF3R* gene that encodes the receptor for colony stimulating factor 3, a cytokine that controls the production and differentiation of granulocytes, was shown to be an important clonal marker of chronic neutrophilic leukemia (CNL), a very rare disease entity among MPNs, based on its high frequency (~90%) in CNLs. *CSF3R* mutations are also gain-of-function mutations, either missense mutations that affect the membrane-proximal extracellular domain of the protein or mutations that lead to the truncation of the protein's cytoplasmic tail. In addition to the diagnostic implications for CNL, *CSF3R* mutation suggests the possibility of a therapeutic response of the mutation-bearing leukemic cells to tyrosine kinase inhibitors. Direct sequencing analysis can detect both forms of *CSF3R* mutation. Table 1 summarizes the updated knowledge of major driver mutations in MPNs.

It is inevitable that the diagnostic criteria that we are using, currently, the 2008 WHO criteria, for the diagnosis of MPNs, will be refined. However, controversies and debate regarding diagnostic criteria still persist; for example, the reliability of the Hb level as a surrogate marker for increased red cell mass has been challenged in the diagnosis of PV. To resolve these challenging issues, proposals for the revision of the WHO diagnostic criteria were recently published [2]. The most remarkable changes are proposed in the diagnostic criteria for PV (Table 2). First, the cut-off value for the Hb level was lowered and Hct level was listed as a new criterion. Based on a study that distinguished *JAK2*-mutated ET from masked PV [5], an Hb level of 16.5 g/dL for men and 16 g/dL for women, or an Hct level of 49% in men and 48% in women, were determined to be the optimal cut-offs. Second, bone marrow morphology was promoted to a major criterion from a minor criterion, validating bone marrow morphology in PV diagnosis. Third, the endogenous erythroid colony formation test was deleted from the list of minor criteria because it is time-consuming and not generally available [6]. Finally, for a rare case of *JAK2*-nonmutated PV, a subnormal erythropoietin level was included as a minor criterion. For ET, the revised criteria include *CALR* or *MPL*, as well as *JAK2* mutation, and one minor criterion (presence of a clonal marker, e.g. abnormal karyotype, or absence of evidence for reactive thrombocytosis) was added for cases in which there are no *JAK2/CALR/MPL* mutations (Table 2). For PMF, a revised criterion also includes *CALR* or *MPL*, as well as *JAK2* mutation. In the absence of *JAK2/CALR/MPL* mutations, the first minor criterion (presence of a clonal marker, e.g. abnormal karyotype, or absence of evidence for reactive BM fibrosis) aims to exclude the possibility of non-clonal bone marrow fibrosis. The second criterion (presence of anemia or palpable splenomegaly) and the third criterion (presence of leukoerythroblastosis or increased LDH level), which includes the lactose dehydrogenase level, reinforce the morphologic expression of PMF [2] (Table 2).

We hope that the revised WHO criteria will enable us to identify patients with MPNs early and efficiently.

Authors' Disclosures of Potential Conflicts of Interest

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

Table 2. 2014 proposed diagnostic criteria for PV, ET, and PMF.

2008 WHO criteria	2014 proposed revision
Polycythemia vera[a]	Polycythemia vera[b]
Major criteria 1. Hb >18.5 g/dL (men) >16.5 g/dL (women) 2. Presence of *JAK2*V617F or *JAK2* exon 12 mutation	*Major criteria* 1. Hb >16.5 g/dL (men), >16 g/dL (women) or Hct >49% (men), >48% (women) 2. BM trilineage myeloproliferation with pleomorphic MK 3. Presence of *JAK2* mutation
Minor criteria 1. BM trilineage myeloproliferation 2. Subnormal serum EPO level 3. Endogenous erythroid colony growth	*Minor criteria* 1. Subnormal serum EPO level
Essential thrombocytemia[c]	Essential thrombocythemia[d]
Major criteria 1. Platelet count $\geq 450\times10^9$/L 2. MK proliferation with large and mature morphology. 3. Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm 4. Demonstration of *JAK2*V617F or other clonal marker or no evidence of reactive thrombocytosis	*Major criteria* 1. Platelet count $\geq 450\times10^9$/L 2. MK proliferation with large and mature morphology. 3. Not meeting WHO criteria for CML, PV, PMF, MDS or other Myeloid neoplasm 4. Presence of *JAK2*, *CALR* or *MPL* mutation *Minor criteria* 1. Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive thrombocytosis
Primary myelofibrosis[e]	Primary myelofibrosis[f]
Major criteria 1. MK proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis 2. Not meeting WHO criteria for CML, PV, MDS or other myeloid neoplasm 3. Demonstration of *JAK2*V617F or other clonal marker or no evidence of reactive BM fibrosis	*Major criteria* 1. MK proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis 2. Not meeting WHO criteria for CML, PV, MDS or other myeloid neoplasm 3. Presence of *JAK2*, *CALR* or *MPL* mutation
Minor criteria 1. Leukoerythroblastosis 2. Increased serum LDH level 3. Anemia 4. Palpable splenomegaly	*Minor criteria* 1. Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive BM fibrosis 2. Presence of anemia or palpable splenomegaly 3. Presence of leukoerythroblastosis or increased LDH level

[a]PV diagnosis requires meeting either both major criteria and one minor criterion or the first major criterion and two minor criteria.
[b]PV diagnosis requires meeting either all three major criteria or the first two major criteria and one minor criterion.
[c]ET diagnosis requires meeting all four major criteria.
[d]ET diagnosis requires meeting all four major criteria or first three major criteria and one minor criterion.
[e]PMF diagnosis requires meeting all three major criteria and two minor criteria.
[f]PMF diagnosis requires meeting all three major criteria or the first two major criteria and all three minor criteria.
Abbreviations: BM, bone marrow; EPO, erythropoietin; MK, megakaryocytes

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