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 JAK2V617F 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
Mutation frequency

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 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.

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

| Gene | Mutation frequency by disease subtype | Mutation hotspot | Mutation type | Molecular methods of mutation detection | WHO diagnostic criteria |
|------|------------------------------------|-----------------|-------------|--------------------------------------|------------------------|
| JAK2 V617F | ~95% in PV | V617F | Gain-of-function missense | qPCR | Included in 2008 |
| JAK2 exon 12 | ~5% in PV | Exon 12 | Gain-of-function missense | Direct sequencing | Inferred in 2008 |
| MPL | ~5% in ET or PMF | M515L/K/A | Gain-of-function missense, small ins/del | Direct sequencing | qPCR, AS-PCR |
| CALR | ~30% in ET or PMF | Exon 9 | Gain-of-function missense, small ins/del | PCR amplicon-sizing analysis | More favorable prognosis than JAK2 mutation carriers |
| CSF3R | ~90% in CNL<sup>b</sup> | - | Gain-of-function missense/non-sense | Direct sequencing | Pending |

*<sup>a</sup>JAK2, MPL, and CALR mutations typically occur in a mutually exclusive manner. <sup>b</sup>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.
Table 2. 2014 proposed diagnostic criteria for PV, ET, and PMF.

| 2008 WHO criteria | 2014 proposed revision |
|-------------------|------------------------|
| **Polycythemia vera**<sup>a)</sup> | **Polycythemia vera**<sup>b)</sup> |
| **Major criteria** | **Major criteria** |
| 1. Hb > 18.5 g/dL (men) | 1. Hb > 16.5 g/dL (men), > 16 g/dL (women) or Hct > 49% (men), > 48% (women) |
| 2. Presence of JAK2V617F or JAK2 exon 12 mutation | 2. BM trilineage myeloproliferation with pleomorphic MK |
| **Minor criteria** | **Minor criteria** |
| 1. BM trilineage myeloproliferation | 1. Subnormal serum EPO level |
| 2. Subnormal serum EPO level | |
| 3. Endogenous erythroid colony growth | |
| **Essential thrombocytemia**<sup>c)</sup> | **Essential thrombocytemia**<sup>d)</sup> |
| **Major criteria** | **Major criteria** |
| 1. Platelet count ≥ 450 × 10⁹/L | 1. Platelet count ≥ 450 × 10⁹/L |
| 2. MK proliferation with large and mature morphology. | 2. MK proliferation with large and mature morphology. |
| 3. Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm | 3. Not meeting WHO criteria for CML, PV, PMF, MDS or other Myeloid neoplasm |
| 4. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive thrombocytosis | 4. Presence of JAK2, CALR or MPL mutation |
| **Minor criteria** | **Minor criteria** |
| 1. Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive thrombocytosis | |
| **Primary myelofibrosis**<sup>e)</sup> | **Primary myelofibrosis**<sup>f)</sup> |
| **Major criteria** | **Major criteria** |
| 1. MK proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis | 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 | 2. Not meeting WHO criteria for CML, PV, MDS or other myeloid neoplasm |
| 3. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive BM fibrosis | 3. Presence of JAK2, CALR or MPL mutation |
| **Minor criteria** | **Minor criteria** |
| 1. Leukoerythroblastosis | 1. Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive BM fibrosis |
| 2. Increased serum LDH level | 2. Presence of anemia or palpable splenomegaly |
| 3. Anemia | 3. Presence of leukoerythroblastosis or increased LDH level |
| 4. Palpable splenomegaly | |

<sup>a)</sup>PV diagnosis requires meeting either both major criteria and one minor criterion or the first major criterion and two minor criteria.

<sup>b)</sup>PV diagnosis requires meeting either all three major criteria or the first two major criteria and one minor criterion.

<sup>c)</sup>ET diagnosis requires meeting all four major criteria.

<sup>d)</sup>ET diagnosis requires meeting all four major criteria or first three major criteria and one minor criterion.

<sup>e)</sup>PMF diagnosis requires meeting all three major criteria and two minor criteria.

<sup>f)</sup>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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