

# Recent advances in diagnosis and treatment of chronic myeloproliferative neoplasms

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## Abstract

The Philadelphia chromosome-negative chronic myeloproliferative neoplasms (MPNs) have recently been the focus of tremendous advances in basic knowledge of disease pathophysiology following the recognition of mutations in *JAK2* and *MPL*. These discoveries also led to refinement of the criteria employed for diagnosis. The prognostic roles of the *JAK2* V617F mutation and of leukocytosis as independent risk factors for thrombosis, which represents the leading cause of death in patients with polycythemia vera and essential thrombocythemia, are supported by retrospective studies. A new risk stratification approach to the patient with primary myelofibrosis allows clinicians to distinguish categories of patients with significantly different expected survival. Finally, new drugs are currently being tested for MPNs, and molecular discoveries could ultimately lead to the development of a specific targeted therapy. Overall, significant advances in diagnosis, prognostication, and treatment have taken place in the last couple of years in the field of MPNs.

## Introduction and context

The Philadelphia chromosome-negative chronic myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [1]. PV and ET are relatively indolent disorders that result in a modest reduction of survival, usually after the first decade from the time of diagnosis; PMF, on the other hand, has a more severe course and a median survival of about 5 years, although some patients experience survival of longer than 10 years. The course of PV and ET is marked by an excess of arterial and venous thrombosis, which represent the principal cause of morbidity and mortality; major hemorrhage, evolution to post-polycythemia vera/post-essential thrombocythemia myelofibrosis (PPV/PET-MF), and transformation to acute myeloid leukemia (AML) also contribute to reduced survival. Causes of death in patients with PMF are related to the consequences of myeloproliferation or bone marrow failure, vascular events, infections, and transformation to AML.

In 2005, a point mutation in exon 14 of the Janus kinase 2 gene (*JAK2* V617F) was discovered in the majority of patients with PV and in about 60% of those with ET or PMF [2-5]. Additional mutations in exon 12 of *JAK2* [6] and at codon 515 of the myeloproliferative leukemia virus oncogene (*MPL*) [7] were reported in a minority of patients with PV or in 5% and 10% of patients with ET and PMF, respectively. These mutant alleles all result in a gain of function due to constitutive activation of tyrosine kinase-dependent cellular signaling pathways, particularly the JAK-signal transducer and activator of transcription (JAK-STAT) pathway [8]. During the search for additional genetic abnormalities in MPNs with the use of high-resolution SNP (single-nucleotide polymorphism) analysis, a region of acquired UPD (uniparental disomy) was identified at chr4q24, where the *TET2* gene maps [9]. Abnormalities in *TET2* are believed to antedate the acquisition of *JAK2* V617F point mutation in most, but not all, cases [9,10]. The precise function of *TET2* is unknown, but the gene might behave as a *bona fide* oncosuppressor. However, it was soon shown that

mutations in *TET2* are found in only 10-15% of MPN patients and are shared by other myeloid malignances, in particular myelodysplastic syndromes and chronic myelomonocytic leukemia [11-14].

Clustering of myeloproliferative disorders in some families has been recognized for some time. Furthermore, a recent epidemiologic study from Sweden found that the risk of developing an MPN is up to seven-fold greater in first-degree relatives of MPN patients than in the general population [15]. The genetic predisposition to MPN, particularly but not exclusively among patients harboring the V617F mutation, has been attributed to a specific haplotype, called 46/1 or GGCC, that is located at chromosome 9 and includes the *JAK2* gene itself [16-19].

Overall, this information has significantly advanced our understanding of the pathophysiology of MPN and, by pointing to mutated kinases as a common mechanism in these disorders, has suggested that tyrosine kinases might represent a valid target for therapy, as exemplified by the efficacy of imatinib and second-generation tyrosine kinase inhibitors in chronic myelogenous leukemia.

## Recent advances

### Diagnosis

One seminal advance has been the revision [20] of the criteria employed for diagnosis that was promoted by the demonstration of an almost universal association of the *JAK2* V617F mutation with PV and an association in about 60% of patients with ET or PMF. According to the 2008 World Health Organization (WHO) classification [21], the presence of any of these mutations constitutes a major criterion for diagnosis. However, the diagnosis of ET or PMF still requires expert evaluation of bone marrow histopathology in order to identify specific morphologic patterns associated with either disease. Another clinical setting in which diagnosis has been significantly improved is represented by the splanchnic vein thromboses that have been found to be associated with the *JAK2* V617F mutation in up to half of cases [22], thus establishing an MPN as the commonest underlying cause [23]. Finally, a consensus statement from the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) proposed uniform criteria for diagnosing the evolution of PV or ET to PPV/PET-MF [24]. Overall, these molecular and clinical advances have made the diagnostic work-up of the commonest MPN more effective and reproducible, a key premise for clinical studies and for the design of trials of innovative drugs.

### Prognosis

The only criteria currently used for risk stratification of PV or ET patients are older age and previous history of

thrombosis. However, several studies, supported by meta-analyses, have documented the association of the *JAK2* V617F mutation with an increased risk of arterial and venous thrombosis in patients with ET (hazard ratio 1.8- to 2.0-fold greater than that of *JAK2* unmutated ET patients) [25], particularly if the mutation is harbored in a homozygous status (i.e., both alleles are mutated) [26]. One study reported that patients with PV who have a high 'allele burden' (i.e., a prevalence of mutated versus normal alleles) are at greater risk for thrombosis [27]. It is also possible that the accumulation of mutated alleles in PV or ET accompanies transformation to PPV/PET-MF [26,28]. On the contrary, PMF patients with lower *JAK2* V617F allele burden are at increased risk of death [29,30]. Thus, evidence that, in addition to diagnostic relevance, a *JAK2* V617F mutated status or a high burden of mutated allele or both have prognostic correlates is being accumulated [31]. On the other hand, *TET2* mutations have not been associated yet with clinical endpoints [32]. Finally, while it is accepted that the occurrence of thrombosis in PV or ET is not directly linked to raised hematocrit or platelet count, leukocytosis has recently been associated with increased risk of major vascular events and eventually could represent a major target of cytoreductive therapy [33].

Identification of PMF patients with expected shorter survival is pivotal for appropriate decision making in regard to hematopoietic stem cell transplantation, which offers the only chance of cure but is still burdened by significant mortality and morbidity. A new and highly discriminatory prognostic system has been devised by the IWG-MRT on the basis of a study of 1054 patients [34]. Variables predicting shortened survival were an age of greater than 65 years, presence of constitutional symptoms, hemoglobin level of less than 100 g/L, leukocyte count of greater than  $25 \times 10^9/L$ , and percentage of circulating blast cells of 1% or greater. When these variables were used, four risk groups that differed significantly in median survival, ranging from 135 to 95, 48, and 27 months, were delineated [34]. These five variables maintained their impact on survival when analyzed as time-dependent covariates during the follow-up of PMF patients, whether younger or older than 65 years [35]. Therefore, prognostic assessment of patients with PMF can be performed anytime during their clinical course by using these criteria and may guide treatment decision.

### Treatment

The central role that alterations in the JAK-STAT pathway play in clinical manifestations of MPN has made targeting *JAK2* an appealing therapeutic goal [36,37]. A number of drugs exhibiting differential inhibitory

activity against JAK family members and eventually on other receptor kinases, such as FGFR (fibroblast growth factor receptor), PDGFR (platelet-derived growth factor receptor), and FLT3 (FMS-like tyrosine kinase 3), are currently in clinical development. Most experience has been in patients with myelofibrosis in phase I/II trials employing INCB018424 (a potent and selective inhibitor of JAK1 and JAK2), TG101348 (a structure-based drug designed to inhibit JAK2 with great selectivity), CEP-701 (a multikinase inhibitor), and XL019 (a selective JAK2 inhibitor whose clinical development has been halted because of neurotoxicity). With some differences, the first results available indicated substantial activity against enlarged spleen and systemic symptoms, whereas the effects on the mutational burden were more variable [38].

While the use of JAK2 inhibitors is ongoing, other important trials have been completed in the last two years. Lenalidomide, an immunomodulating agent, was reported to produce a 20-30% response rate in anemia and splenomegaly, and overall this is similar to the experience with thalidomide and prednisone; myelotoxicity was common [39]. Another study indicated that the combination of lenalidomide and prednisone might be better tolerated, responses were more durable, and a significant reduction of *JAK2* V617F allele burden was reported [40]. However, response rate and quality of response were impressive in myelofibrotic patients harboring the del(5)q abnormality, and lenalidomide should be considered as first-line therapy in this specific subset of patients [41]. A third thalidomide analog, pomalidomide, has been evaluated in a phase II randomized, multicenter, double-blind study. The drug was effective for treatment of anemia in up to 36% of the patients, with minimal hematological and extra-hematological toxicity. No changes in *JAK2* V617F allele burden were observed [42]. Similar results (33% for hepatosplenomegaly and 38% for transfusion-dependent anemia) were reported in a phase II trial with tipifarnib, a farnesyltransferase inhibitor [43].

The role of epigenetic abnormalities in the pathogenesis of MPNs represents a novel field of research [44]. In this regard, a recent paper reported that *JAK2* possesses a nuclear activity and regulates the phosphorylation of histone H3 [45], thereby affecting the expression of target genes, including putative oncogenes such as *lmo2*. Furthermore, TET1, another member of the TET family, has been shown to catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, possibly intervening in the regulation of transcriptional activity [46]. These observations may have therapeutic relevance. However, two similar but independent trials failed to

observe any favorable effect of 5-azacytidine in myelofibrosis [47,48]. On the contrary, ITF2387, a histone deacetylase inhibitor, was reported to have significant activity in the control of hematological abnormalities and systemic symptoms in patients with PV or ET [49].

Interferon (IFN) has been used sporadically in PV and ET for some time. In France, a phase II study of pegylated IFN- $\alpha$ -2a in PV included 40 patients. Eighty-nine percent of the patients presented a mean decrease of the V617 allele of 44%, with complete disappearance of the V617F allele in one patient. The results would confirm the hypothesis that IFN- $\alpha$  preferentially targets the malignant clone in PV [50].

The availability of novel drugs calls for the need to have standardized criteria for assessing response to therapy. While response criteria in patients with PMF were published several years ago [51,52], criteria of response to treatment in PV and ET patients have been published only recently following a European consensus conference [53].

### Implications for clinical practice

Testing of the *JAK2* V617F and, more selectively, *MPL* W515L/K/A or *JAK2* exon 12 mutational statuses has made the diagnosis of MPN more precise than ever before. Therefore, genotyping for those mutations is mandatory in the work-up of a patient suspected of having an MPN. Nowadays, diagnoses are expected to strictly fulfill the 2008 WHO criteria. Also, the IWG-MRT criteria for the diagnosis of PPV/PET-MF should be routinely adopted in clinical practice. Genotype-phenotype associations have been identified; however, quantitative methods for measuring *JAK2* V617F allele burden are specialized tools that are still best used within clinical trials. After all, the decision to treat ET or PV patients based on the presence of the mutation, a high mutated allele burden, or leukocytosis as surrogate markers of increased thrombotic risk deserves prospective validation and cannot be routinely recommended outside clinical trials.

It is now time to start assuming that the clinical course of MPNs may be improved by therapy and the disease possibly cured. Molecular discoveries have made the development of *JAK2* tyrosine kinase inhibitors an attractive therapeutic goal, and molecules that inhibit *JAK2* kinase are being evaluated with measurable results in the clinical setting. Actually, the rapid and marked improvement in splenomegaly and quality of life observed in myelofibrotic patients treated with INCB018424 or TG101348 has never been seen before with any drug and *per se* justify enthusiasm. Novel

immunomodulatory agents may be better tolerated and more effective than thalidomide in the setting of myelofibrosis. Drugs acting on epigenetic gene regulation, either alone or eventually in combination, hold some promise. Finally, while it is noteworthy that IFN proved able to induce clonal remission in patients with PV, larger studies are needed to define the proportion of patients achieving such a goal as well as the long-term treatment tolerability of pegylated formulations. However, whether aiming at molecular remission is a valuable clinical endpoint for the long-term management of PV or ET, as it might be for myelofibrosis, still requires demonstration.

**Abbreviations**

AML, acute myeloid leukemia; ET, essential thrombocytopenia; IFN, interferon; IWG-MRT, International Working Group for Myelofibrosis Research and Treatment; JAK, Janus kinase; JAK-STAT, JAK-signal transducer and activator of transcription; MPL, myeloproliferative leukemia virus oncogene; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; PPV/PET-MF, post-polycythemia vera/post-essential thrombocytopenia myelofibrosis; PV, polycythemia vera; WHO, World Health Organization.

**Competing interests**

The authors declare that they have no competing interests.

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