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Advances in Understanding and Management of Myeloproliferative Neoplasms

Alessandro M. Vannucchi, MD[1]; Paola Guglielmelli, MD[2]; Ayalew Tefferi, MD[3]

Abstract

According to the 2008 World Health Organization classification system for hematologic malignancies, the myeloproliferative neoplasms (MPN) include chronic myelogenous leukemia, polycythemia vera, essential thrombocythemia, primary myelofibrosis, mastocytosis, chronic eosinophilic leukemia-not otherwise specified, chronic neutrophilic leukemia, and "MPN, unclassifiable." All of these clinicopathologic entities are characterized by stem cell-derived clonal myeloproliferation, and their phenotypic diversity is ascribed to the occurrence of distinct oncogenic events. In the last 4 years, new *JAK2* and *MPL* mutations have been added to previously described *ABL* and *KIT* mutations as molecular markers of disease in MPN. These discoveries have markedly simplified the approach to clinical diagnosis and have also provided molecular targets for the development of small-molecule drugs. In the current article, the authors provide a clinically oriented overview of MPNs in terms of their molecular pathogenesis, classification, diagnosis, and management. **CA Cancer J Clin 2009;59:171-191. ©2009 American Cancer Society, Inc.**

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Introduction

In 1951, William Dameshek[1] introduced the term "myeloproliferative disorders (MPD)" to encompass polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF),[2] chronic myelogenous leukemia (CML), and Di Guglielmo's syndrome (erythroleukemia). His proposal was based on similarities in the clinical phenotype of these disorders and on the hypothesis that a generalized proliferation of bone marrow cells, due to some unknown stimuli, was the underlying cause. The association of the Philadelphia (Ph^1)-chromosome with CML in 1960,[3] and the subsequent recognition of erythroleukemia as a variant of acute myeloid leukemia (AML), distinguished the other three disorders as "classic" Ph^1-negative MPD.[4]

The first systematic attempt to classify MPD and MPD-like clinicopathologic entities was undertaken by the World Health Organization (WHO) committee for the classification of hematologic malignancies.[5] According to the 2001 WHO classification system, CML, PV, ET, and PMF were included under the category of "chronic myeloproliferative diseases" (CMPD). The CMPD category also included other "nonclassic" MPD-like disorders such as chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia/hypereosinophilic syndrome (CEL/HES), and "unclassified CMPD." The identification of *BCR-ABL* as a CML-specific genetic event, in the context of CMPD, has facilitated accurate molecular diagnosis and effective targeted therapy. The lack of

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knowledge, until recently, on specific genetic defects in the other *BCR-ABL*-negative classic CMPDs necessitated that diagnosis rest on a combination of bone marrow histology and a few clinical and laboratory findings to distinguish clonal from reactive myeloproliferation and one CMPD from another.[6]

The last 4 years have witnessed fundamental advances in understanding the molecular pathogenesis of classic *BCR-ABL*-negative CMPD, capped by the discovery of specific molecular abnormalities associated with PV, ET, and PMF.[7] As a result, WHO diagnostic criteria have been revised,[8] and the term "CMPD" has been changed to "myeloproliferative neoplasms (MPN)."[8] It is hoped that newly discovered mutations will also facilitate development of targeted therapy. At the same time, large clinical studies continue to provide practically useful clinical information.

The current review has two main objectives. The first is to provide a general overview of MPN including their molecular pathogenesis and updated WHO classification. The second objective is to describe in more detail the criteria for diagnosis, risk stratification, and management of patients with the classic *BCR-ABL*-negative MPN including PV, ET, and PMF.

TABLE 1. Recurrent Molecular Abnormalities Associated with Myeloproliferative Neoplasms

GENETIC ABNORMALITY	DISEASE	FREQUENCY
BCR-ABL	Chronic myelogenous leukemia	≅99%
*JAK2*V617F	Polycythemia vera	>95%
	Essential thrombocythemia	≅60%
	Primary myelofibrosis	≅60%
	MPN, unclassifiable	≅20%
	Refractory anemia with sideroblasts and thrombocytosis (RARS-T)	≅50%
JAK2 exon 12	Polycythemia vera	≅2%
*MPL*W515L/K*	Primary myelofibrosis	≅8%
	Essential thrombocythemia	≅8%†
Involving *PDGFRA*	Myeloid neoplasms with eosinophilia	Unknown
	Mast cell disease	Unknown
Involving *PDGFRB*	Myeloid neoplasms with eosinophilia	Unknown
Involving *FGRF1*	Myeloid neoplasms with eosinophilia	Unknown
Involving *KIT* (D816V as the most frequent)	Mast cell disease	Unknown

MPN indicates myeloproliferative neoplasm.
*Other infrequent mutations, such as W515A or S505N, have been reported.
†Calculated on *JAK2*V617F-negative patients.

Molecular Basis of Myeloproliferative Neoplasms

Apart from the *BCR/ABL* rearrangement in CML, originated by a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34; q11),[9] or the chimeric *FIP1L1-PDGFRA* mRNA in some forms of eosinophilia,[10] and *kit* mutations in cases with systemic mastocytosis,[11] information concerning molecular abnormalities of MPN has been scanty until 2005, when a Janus kinase 2 mutation (*JAK2*V617F) was discovered in the majority of patients with PV and in 50% or fewer of those with ET or PMF.[12-15] In the following 2 years, additional mutations in *JAK2*[16] and *MPL*[17,18] were reported (Table 1). These different mutant alleles all result in a gain of function due to the constitutive activation of tyrosine kinase-dependent cellular signaling pathways, particularly of the JAK-STAT pathway.[19,20] Overall, this would suggest that mutated kinases represent a common pathogenetic mechanism in these disorders and that, as exemplified by the efficacy of the tyrosine kinase inhibitor imatinib in CML, they could represent valid targets for therapy.[21,22]

Members of the Janus kinase family (JAK1, JAK2, JAK3, and tyrosine kinase 2-Tyk2) are named after the Roman god with two faces, meaning ending and beginning, because they contain two symmetrical kinase-like domains: the C-terminal JAK homology 1 (JH1) domain possesses tyrosine kinase function, whereas the immediately adjacent JH2 domain is enzymatically inactive, but it is credited with negatively regulating the activity of JH1.[23,24] Ordinarily, JAKs are associated in an inactive state to the cytoplasmic tail of type 1 or type 2 cytokine receptors (eg, erythropoietin receptor, EpoR; thrombopoietin receptor, MPL; granulocyte colony-stimulating factor receptor, G-CSFR; and interferon-gamma receptor,

to name a few). After the engagement of the receptor by corresponding ligand, JAK undergoes a conformational change and becomes activated via phosphorylation of key tyrosine residues. In turn, phosphorylated JAKs mediate phosphorylation of tyrosine residues of the cytoplasmic domain of the receptors and create a docking site for the recruitment of several proteins, ultimately leading to activation of the signal transducer and activator of transcription (STAT), the mitogen-activated protein (MAP) kinase, and the phosphatidylinositol 3-kinase-AKT (PI3K-AKT) pathways[25] (Fig. 1A). Activated STATs dimerize and translocate to the nucleus where they regulate transcription after binding to specific consensus sequences in the promoter regions of several target genes (Fig. 1A). The entire process is tightly controlled at multiple levels by protein tyrosine phosphatases, suppressors of cytokine signaling (SOCS), and protein inhibitors of activated STAT.[26-29] JAK2, and possibly other JAKs, is also involved in the expression of cognate receptors EPOR and MPL at the cell surface by acting as a chaperon and protein stabilizer.[30,31]

The *JAK2*V617F mutation is a somatically acquired G to T nucleotide shift at position 1849 in exon 14 that results in a valine to phenylalanine substitution at codon 617; the mutation is located in the JH2 pseudo-kinase domain and is believed to result in the loss of auto-inhibitory control of JAK2 (Fig. 1B). As a consequence, mutated JAK2 is in a constitutively phosphorylated state, independent from the binding of ligand to its receptor; in fact, when the mutation is introduced into cytokine-dependent cell lines it results in a cytokine-independent growth of the cells and their hypersensitivity to cytokines,[13,14] mimicking the in vitro growth pattern of hematopoietic progenitors from MPN patients. In particular, the gain of function of mutated JAK2 provides a mechanistic explanation for the phenomenon of endogenous erythroid colony formation (EEC),[33,34] ie, the capacity of erythroid progenitors to spontaneously produce hemoglobinized colonies in vitro in the absence of added erythropoietin, a hallmark of PV and other classic MPNs. Furthermore, transplantation of *JAK2*V617F mutated cells induced a PV-like phenotype in recipient mice,[13,35-38] accompanied by leukocytosis of a different extent and eventually followed by changes suggestive of myelofibrotic transformation.[35-38] More recently, by manipulating expression levels of the V617F allele, mice with an ET-like phenotype were also generated in the presence of low levels of mutated JAK2.[39] Over-

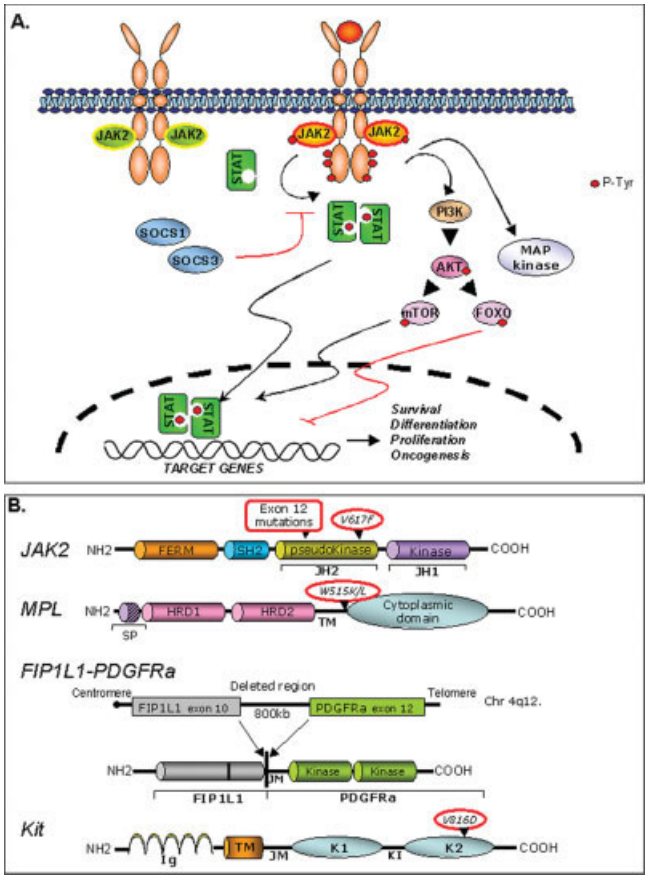

FIGURE 1. **(A)** In normal hematopoietic cells, signaling is initiated when cytokines bind to and activate their cell surface type-1 receptors, which have molecules of JAK2 associated to the cytoplasmic domains. After ligand engagement (the pathway activated by EPO bound to the EPOR is herein schematized) the receptor-associated JAKs become activated through auto-phosphorylation and on turn phosphorylate tyrosine residues in the receptor cytoplasmic tail. The receptor phosphotyrosines serve as docking sites for the recruitment of inactive cytoplasmic STAT monomers through interaction with their SH2 domain. JAK-mediated phosphorylation of tyrosine residues on the receptor-bound STAT monomer induces STATs dimerization. The activated dimers translocate to the nucleus, where they bind to specific DNA-responsive elements in the promoters of target genes and thereby induce unique gene expression program(s). Activation of JAK2 pathway also results in the recruitment and activation of MAPk signaling proteins and AKT/mTOR/FOXO pathway that transmit signals for survival, proliferation, and differentiation of erythroblastic progenitors; JAK2-independent activation of these pathways might also occur. Negative feedback mechanisms are normally mediated, among other regulators, by SOCS proteins. These complex signals are autonomously activated, in the absence of binding of the cytokine to its receptor, when JAK2 is mutated (JAK2V617F or activating mutations in exon 12) or the receptor itself is mutated (as is the case of W515L/K mutation of MPL receptor). **(B)** Schematic representation of the most common genetic abnormalities associated with MPN. For details, please refer to text.
STAT indicates signal transducer and activator of transcription; AKT, protein kinase B, PKB; FOXO, forkhead transcription factors; PI3K, phosphatidylinositol-3′-kinase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; SOCS, suppressor of cytokine signaling; *JAK2*, Janus kinase 2 gene; *MPL*, thrombopoietin receptor gene; *FIP1L1-PDGFRA*, fusion gene of Fip1-like 1 with platelet-derived growth factor receptor alpha; *kit*: stem cell factor (SCF) receptor gene; FERM: 4-point-1, Erzin, Radixin, Moesin JAK2 amino-terminal domain; JH1, JAK homology 1 (active tyrosine kinase) domain; JH2, JAK homology 2 (catalytically inactive pseudokinase) domain; SH2, SRC homology 2 domain; HRD1, HRD2, Hematopoietin/cytokine receptor domain 1 (negative regulatory domain) or domain 2 (ligand binding region); SP, signal peptide; TM, trans-membrane domain; JM, juxtamembrane domain; Ig, Immunoglobuline-like repeat; K1, Kinase domain 1; KI, 76 amino acids kinase insert domain; K2, kinase domain 2.

all, these models indicated that the *JAK2*V617F mutation is sufficient to induce a MPN-like phenotype in mice and suggested that the level of mutated allele may influence disease phenotype.[40]

Mutational frequency of *JAK2*V617F is estimated to be more than 95% in PV, 60% in ET or PMF, 40% to 50% in refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T),[41] whereas it is very rare in AML or MDS.[42-45] In most patients with PV or PMF, as opposed to a minority of those with ET, the mutation is harbored in a homozygous state, which is accomplished by mitotic recombination.[12-15] In general, the highest V617F allele burden, that is the level of mutated allele relative to normal allele in a cell suspension such as granulocytes, is found in patients with PV followed by PMF and ET[46,47]; however, such variability in the allele burden does not represent a sufficient criterion for distinguishing among different clinical entities, nor does it satisfactorily help to explain the apparent paradox of "one mutant allele-different clinical phenotypes." In fact, how a single V617F mutation can be the basis of different clinical disorders, as in the classic MPN, is still unclear. Interestingly, single nucleotide polymorphisms (SNPs) in *JAK2* have been associated preferentially with the diagnosis of PV,[48] supporting the contribution of inherited host genetic characteristics to MPN phenotypic variability. Regardless, there is evidence to suggest that *JAK2*V617F may not be the initial clonogenic event in MPN and that a "pre-*JAK2*" mutated cell may exist.[49,50] In support of this is also a finding that leukemic blasts in patients who evolve to AML from a pre-existing *JAK2*V617F-positive MPN are often negative for the *JAK2*V617F mutation.[51,52] Conversely, *JAK2*V617F, or other *JAK2* mutations, are likely a necessary component of the PV phenotype because they are detected in virtually all patients with the disease[53] and are sufficient to reproduce the phenotype in mice. In summary, *JAK2*V617F mutation is integral to the classic MPN, but its exact hierarchical position in pathogenesis and its role in phenotypic variability remain to be clarified. After all, one could conclude that PV, ET, and PMF are separate diseases or different presentations or different phases of a unique disease. It has been suggested that the phenotype of patients with *JAK2*V617F-positive ET resembles "forme fruste" of PV.[54]

In patients with a clinical picture suggestive of PV and who were found to be negative for the *JAK2*V617F mutation, several genetic abnormalities (ie, mutations, deletions, insertions) have been detected in a short region of *JAK2* exon 12 (Fig. 1B).[16,55] These mutations, which probably account for less than 2% of patients with PV,[55] affect autonomous cell proliferation and differentiation in a fashion similar to that of the V617F allele.[16]

Another recurrent molecular abnormality of MPN is represented by somatic mutations at codon 515 of *MPL*,[17,18] which, as is the case with *JAK2*V617F, involve early myeloid and lymphoid progenitors.[56-58] MPL (named after myeloproliferative leukemia virus oncogene homolog) is the receptor for the cytokine thrombopoietin (Tpo) and is highly expressed in early hematopoietic progenitors and in cells of the megakaryocytic lineage.[59] The two most common *MPL* mutations, which are located in the cytoplasmic juxtamembrane portion, are represented by W515L (a tryptophan to leucine substitution) and W515K (a tryptophan to lysine substitution; Fig. 1B). They have been detected in 5% to 11% of patients with PMF[17,18,60] and in up to 9% of *JAK2*V617F-negative cases of ET.[61,62] Other unusual MPL mutations (eg *MPL*W515S, W5151A, and *MPL*S505N, initially discovered in association with inherited familial thrombocytosis) have also been reported.[63] *MPL*W515L induced both cytokine-independent growth and Tpo hypersensitivity in cell lines, resulting in constitutively activated JAK-STAT/ERK/Akt signaling pathways,[64] and caused a PMF-like disease in mice.[17] At variance with the *JAK2*V617F transplantation model, the disease induced by *MPL*W515L was characterized by a rapidly fatal course, marked thrombocytosis, leukocytosis, hepatosplenomegaly, and bone marrow fibrosis, all reminiscent of PMF.[17] Interestingly in some patients, multiple *MPL* mutations or the coexistence with *JAK2*V617F allele were described.[60,62,65]

The gene encoding for the receptor of platelet-derived growth factor A (PDGFRA) is involved in at least four different genetic abnormalities associated with eosinophilia.[66] The most frequent and best characterized abnormality is due to a karyotypically occult microdeletion at chromosome 4q12, where *PDGFRA* is located, resulting in a chimeric *FIP1L1-PDGFRA* fusion gene (Fig. 1B).[10] The latter encodes for an aberrantly activated tyrosine kinase as the consequence of disruption of the autoinhibitory activity encoded by *PDGFRA* exon 12, where the breakpoint is located; this constitutively active ty-

rosine kinase drives autonomous eosinophil progenitor proliferation,[67] possesses transforming properties in vitro, and induces a myeloproliferative disorder with extensive eosinophil proliferation when expressed in transplanted mice.[68] The fusion gene has been demonstrated at the level of hematopoietic stem cell compartment.[69] Also the Beta type of PDGFR has been reported as being involved in rearrangements[70] associated with imatinib-responsive eosinophilia.[71] The *PDGFRB* is located at chromosome 5q31-32 and may fuse with different partners. One of the most common is the *ETV6/TEL* gene on chromosome 12p13, which encodes for a transcription factor with nonredundant roles in normal hematopoiesis.[72] The fusion protein constitutively activates the cellular pathways normally associated with PDGFRB signaling[73] and has transforming properties when expressed in cell lines.

A D816V mutation located in the catalytic domain of the tyrosine kinase receptor c-Kit occurs in systemic mastocytosis (Fig. 1B).[11,74] c-Kit is the receptor for stem cell factor, a key cytokine involved in the generation and differentiation of mast cells from primitive hematopoietic progenitors; it is encoded by *kit*, located at chromosome 4q12. Additional activating *kit* mutations other than D816V have also been described in SM, acute leukemia,[75] gastrointestinal stromal cell tumors (GIST), and germ cell tumors.[76] The D816V and other homologous mutations induce growth factor independent growth and cell differentiation in mast cell lines through activation of STAT5/PI3K/AKT signaling pathways and a phenotype resembling human SM in murine models.[77]

Classification of Myeloproliferative Neoplasms

The 2008 WHO classification for myeloid neoplasms, which incorporates novel information derived from molecular discoveries in *BCR-ABL* negative "classic" myeloproliferative states and clonal eosinophilic disorders, includes five major entities (Table 2)[8] as follows: the Acute Myeloid Leukemia (AML) and the Myelodysplastic Syndromes (MDS) with their different subtypes, whose listing is outside the scope of this review; the Myeloproliferative Neoplasms (MPN); the category of overlapping Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN); and the Myeloid Neoplasms associated with eosinophilia and specific molecular abnormalities. AML is defined by the presence of either ≥20% blast cells in the bone marrow and/or peripheral blood or certain characteristic cytogenetic abnormalities.[78] The MDSs are recognized and distinguished from MPN primarily on the basis of the presence of trilineage dyshematopoiesis in the absence of monocytosis in both bone marrow and peripheral blood.[78]

A "nontrivial" formal modification in the 2008 WHO classification has been the substitution of the attribute "neoplasm" for "disease". In fact, notwithstanding the analysis of the X chromosome inactivation pattern in informative females and other cytogenetic and/or molecular findings that established both "classic" and "nonclassic" myeloproliferative disorders as being clonal stem cell disorders,[79-89] and the finding that evolution to AML is part of their natural history,[90] the neoplastic nature of these conditions

TABLE 2. The 2008 World Health Organization Classification for Myeloid Neoplasms

1. Acute myeloid leukemia (AML) and related precursor neoplasms
2. Myelodysplastic syndromes (MDS)
3. Myeloproliferative neoplasms (MPN)
3.1. Chronic myelogenous leukemia (CML), *BCR-ABL1* positive
3.2. Polycythemia vera (PV)
3.3. Essential thrombocythemia (ET)
3.4. Primary myelofibrosis (PMF)
3.5. Chronic neutrophilic leukemia (CNL)
3.6. Chronic eosinophilic leukemia, not otherwise classified (CEL-NOS)
3.7. Mastocytosis
3.8. Myeloproliferative neoplasm, unclassifiable (MPN-u)
4. Myelodysplastic/Myeloproliferative neoplasms (MDS/MPN)
4.1. Chronic myelomonocytic leukemia (CMML)
4.2. Juvenile myelomonocytic leukemia (JMML)
4.3 Atypical chronic myeloid leukemia, *BCR-ABL1* negative
4.4. Myelodysplastic/myeloproliferative neoplasm, unclassifiable
4.5. Refractory anemia with ring sideroblasts associated with marked thrombocytosis
5. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*
5.1. Myeloid and lymphoid neoplasms associated with *PDGFRA* rearrangement
5.2. Myeloid neoplasms with *PDGFRA* rearrangement
5.3. Myeloid and lymphoid neoplasms with *FGFR1* abnormalities

From Tefferi A and Vardiman JW.[136]

has been mostly dismissed until recently. This belief has most likely represented one of the reasons for the traditionally poor interest in these neoplasms by cancer surveillance programs, agencies granting research support, or pharmaceutical companies.

The four "classic" MPNs (ie, CML, PV, ET, and PMF) should be distinguished from the other "nonclassic" MPNs, which include chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia-not otherwise specified (CEL-NOS), systemic mastocytosis (SM), and unclassifiable forms of MPN.[91] CML presents very unique characteristics, and it will not be further discussed herein; recent excellent reviews on molecular and therapeutic issues have been published.[92-94]

Chronic Neutrophilic Leukemia

CNL is a rare disorder of elderly people characterized by neutrophilic leukocytosis (greater than 25×10^9/L) made up of greater than 80% mature granulocytes, splenomegaly, and an absence of the Philadelphia chromosome/*BCR-ABL* fusion gene. Bone marrow biopsy reveals hyperplasia of granulocytic lineage without involvement of other series, and there is an absence of fibrosis or myelodysplastic features. Given the potential for evolution to acute leukemia or progressive refractory leukocytosis, allogeneic stem cell transplantation may be appropriate for younger patients.[95,96]

Chronic Eosinophilic Leukemia and Hypereosinophilic Syndrome

Patients who have a persistent absolute eosinophil count of at least 1.5×10^9/L, after exclusion of reactive eosinophilias or other hematologic disorders, suffer from one of the different forms of primary eosinophilia.[97,98] Many patients with nonclonal forms of eosinophilia fall within the category of "idiopathic" hypereosinophilia; the possibility of a T-cell mediated eosinophilia, generally via increased levels of interleukin-5, can be ruled out with adequate studies of T-cell immunophenotyping and T-cell receptor antigen gene rearrangement.[99] Conversely, finding a cytogenetic or molecular abnormality would indicate a clonal, myeloproliferative, eosinophilic disorder.[98] Diagnosis of CEL not otherwise (molecularly) specified rests on the demonstration of a cytogenetically abnormal proliferation of eosinophilic precursors with a myeloblast count of 5% to 19% in the bone marrow or greater than 2% in peripheral blood, usually accompanied by evidence of organ damage.[97] However, because of intrinsic difficulties in establishing the presence of a clonal disorder when the most frequent molecular abnormalities associated with eosinophilia are lacking (see below), it is likely that many forms of CEL-NOS actually fall improperly within the idiopathic hypereosinophilic syndrome (HES) category.[100] Documentation of target organ damage is necessary for a patient to be considered as suffering from HES. Clinical manifestations are related to eosinophilic infiltration of target tissues and may range from almost asymptomatic disease to fatal endomyocardial tissue fibrosis. Bone marrow biopsy reveals eosinophilia without involvement of other cell lines, absence of immature myeloid cells or dysplasia, and a normal number of mast cells. Therapy is based on corticosteroids as first-line therapy, interferon-alpha, or hydroxyurea in refractory or steroid-dependent patients; some patients may respond to imatinib.[101] Use of monoclonal antibodies to interleukin-5 (mepolizumab)[102] or CD52 (the receptor for interleukin 2; alemtuzumab)[103] has produced appreciable results in refractory cases.[66]

Mast Cell Disease

Mast cell disease, which is defined by tissue infiltration by abnormal mast cells, can be broadly classified into cutaneous mastocytosis (CM) and systemic mastocytosis (SM); the latter might have an indolent or an aggressive clinical course depending on the absence or presence, respectively, of impaired organ function.[104] Life expectancy is nearly normal in indolent forms of SM but is significantly shortened in aggressive SM. The bone marrow is almost universally involved in SM and is characterized by dense, multifocal aggregates of morphologically and immunophenotypically abnormal mast cells, preferentially in a perivascular location, and is often accompanied by increased eosinophils. Serum levels of tryptase are typically high and represent a clinically useful disease-related marker. By using adequately sensitive molecular techniques (such as allele-specific polymerase chain reaction [PCR] amplification of DNA) and mast cell-enriched sources (such as bone marrow aspirate or biopsied lesional material), the *kit* D816V mutation is detected in virtually all patients with

SM.[105] In addition to its diagnostic value, clinical relevance of searching the D816V *kit* mutation lies in the almost universally reported refractoriness of mutated patients to imatinib.[106] Conversely, rare patients with other mutations that are located in the c-Kit juxtamembrane portion may respond to treatment with imatinib. Treatment of systemic mastocytosis is highly individualized and largely palliative, aiming to prevent or reduce symptoms due to mast cell degranulation or organ infiltration. Therapeutic options are represented by interferon-alpha or cytotoxic drugs, such as cladribine, but clinical responses are limited.[107]

Myelodysplastic/Myeloproliferative Neoplasms

MDS/MPN neoplasms are defined by simultaneous presence of both myelodysplastic and myeloproliferative features that exclude them from being categorized as either MDS or MPN alone.[108] MDS/MPN neoplasms comprise chronic myelomonocytic leukemia (CMML), juvenile MML (of pediatric interest and, thus, not further discussed here), atypical CML, and unclassified MDS/MPN. The clinical and hematologic presentation of MDS/MPN is pleomorphic, with cytopenia and dysplasia of any cell lineage eventually becoming associated with elevated leukocyte or platelet count. Symptoms may be attributed to cytopenias (anemia, infections, hemorrhage) and/or to myeloproliferation (organomegaly, systemic symptoms, cardiovascular events).[91] The molecular basis of these disorders is largely unknown, apart from the involvement of ras pathway with mutations of *RAS*[109] in CMML and *PTPN11*[110] mutations in JMML, or the uncommon presence of *JAK2*V617F mutation.[111] Therefore, diagnosis relies on a combination of hematological, clinical, and histological criteria.

The typical manifestation of CMML is peripheral blood monocytosis greater than 1×10^6/L and a percentage of monocytes in the white blood cell count of greater than 10%. Monocytes may or may not display signs of dysplasia, but the percentage of immature monocytes (promonocytes) and monoblasts in peripheral blood is less than 20%. Both monocytic and granulocytic hyperplasia are found in the bone marrow with a total blast count of less than 20%; signs of erythroid and megakaryocytic dysplasia are variably present. Random cytogenetic abnormalities can be discovered in 20% to 40% of these patients. The prognosis is unfavorable with a median survival of only 2-4 years; a major determinant of survival is the percentage of blood and bone marrow blasts.[91] Hypomethylating agents like decitabine and azacitidine are now approved for treating CMML.[112] Response rate varies from 10% to 30%, depending on the drug and the schedule used, with best results reported for decitabine when a high-dose intensity regimen is used.[113-115] Treatment is well tolerated with relatively few nonmyelosuppressive complications and has become a valuable therapeutic option for a disease where just a few years ago the standard of care was merely supportive.

Atypical CML is a rare, aggressive disorder with a median survival of 1-2 years and usually affects elderly patients. It presents features typical of classic CML, but unlike classic CML, it is *BCR/ABL* negative and displays manifest signs of dysgranulopoiesis with nuclear aberrations and cytoplasmic hypogranulation.[108] *JAK2*V617F mutation is absent.[116] The bone marrow is hypercellular; dysplasia of myeloid lineage with less than 20% blasts is a constant feature, whereas other lineages are variably involved. The disease terminates in AML in up to 40% of these patients.

Refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) is a rare syndrome characterized by anemia with dyserythropoiesis and ring sideroblasts in the bone marrow, associated with thrombocytosis and increased number of large megakaryocytes. These morphologic features of megakaryocytes are distinct from the appearance typically associated with the 5q- abnormality.[117] A high proportion of patients have the *JAK2*V617F mutation,[118] although a few harbor the *MPL* mutation.[119] RARS-T is a disease with a relatively good prognosis,[120] and it shares many aspects with classic MPN.

Finally, when myelodysplastic and myeloproliferative features simultaneously present in bone marrow aspirate do not fit into any of the previous categories, and after any known molecular or cytogenetic abnormality is excluded, the disorder is defined as MDS/MPN unclassifiable, with a comment describing the atypical features.[108]

Myeloid Neoplasms Associated with Eosinophilia and Specific Molecular Abnormalities

Both the alpha (*PDGFRA*) and beta *(PDGFRB*) types of platelet-derived growth factor receptor (*PDGFR*) genes may be involved in genetic abnormalities associated with eosinophilia.[121] Eosinophilia associated with *FIP1L1-PDGFRA* rearrangement has a strikingly male predominance. In addition to an expanded eosinophilic lineage, the bone marrow often contains an increased number of mast cells that, together with findings of raised serum levels of tryptase, sometimes make problematic the differential diagnosis with SM.[122] However, at variance with *kit* D816V-mutated forms of SM, presence of the *FIP1L1-PDGFRA* mutation reliably predicts hematologic and molecular remission when imatinib is used at doses lower than those used for CML (100 mg daily is generally efficacious).[123] The rate of complete molecular response may be as high as 95%, and a prospective multicenter study showed it to be stable and durable during a median follow-up of 25 months but to be dependent on treatment continuation. In three patients who discontinued imatinib, molecular negativity was lost and then regained after imatinib was resumed.[124] A definitely lower proportion of patients with imatinib-responsive eosinophilia have rearrangements involving the *PDGFRB* gene. Finally, translocations involving the fibroblast growth factor receptor-1 gene (*FGFR1*), which is located at chromosome 8p11, and several different gene partners are at the basis of the "8p11 myeloproliferative syndrome".[125] This is also called "stem cell leukemia/lymphoma syndrome" because of the clinical phenotype that is characterized by features of both lymphoma and eosinophilic myeloproliferation. The disease results from constitutive activation of the tyrosine kinase domain of FGFR1 after its juxtaposition with any partner gene. Prognosis is very poor with most patients progressing to overt AML or lymphoblastic lymphoma with 1-2 years of diagnosis.[126]

The "Classic" Myeloproliferative Neoplasms

Among classic MPNs, PV and ET are relatively indolent disorders,[127] resulting in a modest reduction of lifespan compared with a control population; however, most patients ultimately suffer from one or more severe and potentially fatal complications directly attributable to the disease. Conversely, PMF has a severe course in most cases, and survival is significantly affected. The three clinical entities share several common features,[6] such as their origin in a multipotent hematopoietic stem cell, a relatively normal cellular maturation, a striking overlap in clinical presentation (apart from PMF, which has its own peculiar manifestations), and in cases of PV and ET, the propensity to evolve into post-polycythemic or post-thrombocythemic myelofibrosis (or less frequently each into the other), and the possibility to transform into AML.[90]

Epidemiology

Classic MPNs are among the most frequent hematologic neoplasms, usually affecting the adult elderly population; however, they can also be found in children, and in this instance, they raise specific diagnostic and management issues[128,129] that are beyond the scope of this review. A recent study,[130] based on the North American Association of Central Cancer Registries (NAACCR) encompassing 82% of total US population, reported an average 2001-2003 annual age-adjusted incidence rate of 2.1 per 100,000 and estimated that there were 6,328 new cases in the total US population in 2004. Furthermore, because of their relatively smooth clinical course, it is likely that many classic MPN cases actually go undetected or are not reported to registries. Advanced age, male sex, and white race were identified as risk factors. Among individuals aged 80 years or older, the rate was as high as 13.3 per 100,000. Familial clustering of these disorders is known, and even before the discovery of *JAK2*V617F mutation, [131] this observation led to a suggestion of predisposition allele(s).[132] This hypothesis gained substantial support from a large study recently completed in Sweden.[133] Relatives of patients with MPN had a 5.7 relative risk (RR) of having PV, an RR 7.4 for ET, and an RR of 7.5 for unclassified forms of MPN, together with a borderline increased RR of CML. The higher risk observed among siblings would suggest a model of recessive inheritance, although whether presentation of disease occurs at an even younger age than in parents is debatable.[133,134] Accordingly, thorough investigation of family history is mandatory in the initial workup of patients with classic MPN, and appropriate counsel-

TABLE 3. 2008 WHO Diagnostic Criteria for "classic" MPN

CRITERIA	POLYCYTHEMIA VERA	ESSENTIAL THROMBOCYTHEMIA	PRIMARY MYELOFIBROSIS
Major criteria	**1.** Hgb >18.5 g/dL (men) or >16.5 g/dL (women) **or** Hgb or Hct > 99th percentile of reference range for age, sex, or altitude of residence **or** Hgb >17 g/dL (men) or >15 g/dL (women) if associated with a documented and sustained increase of ≥2 g/dL from baseline that cannot be attributed to correction of iron deficiency **or** elevated red cell mass >25% above mean normal predicted value **2.** Presence of *JAK2*V617F or similar mutation	**1.** Sustained platelet count ≥450 x 10^9/L **2.** BM 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 the WHO criteria for PV, PMF, CML, or MDS or other myeloid neoplasm **4.** Demonstration of *JAK2*V617F or other clonal marker **or** no evidence of reactive thrombocytosis	**1.** Megakaryocyte proliferation and atypia* accompanied by either reticulin and/or collagen fibrosis **or** In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (ie, pre-fibrotic cellular-phase disease) **2.** Does not meet WHO criteria for CML, PV, MDS, or other myeloid neoplasm **3.** Demonstration of *JAK2*V617F or other clonal marker **or** no evidence of reactive marrow fibrosis
Minor criteria	**1.** BM showing hypercellularlity for age and trilineage growth (panmyelosis)	—	**1.** Leukoerythroblastosis
	2. Subnormal serum Epo level		**2.** Increased serum LDH
	3. EEC growth		**3.** Anemia
	—		**4.** Palpable splenomegaly
Diagnostic combinations	Both major criteria + 1 minor criterion **or** first major criterion + 2 minor criteria	All 4 criteria must be met	All 3 major criteria + 2 minor criteria

WHO indicates World Health Organization; MPN, myeloproliferative neoplasm; CML, *BCR-ABL1* chronic myelogenous leukemia; PV, polycythemia vera; PMF, primary myelofibrosis; MDS, myelodysplastic syndrome; BM, bone marrow biopsy specimen; Epo, erythropoietin; EEC, endogenous erythroid colonies; LDH, lactate dehydrogenase.
*Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering.

ing should be provided. The coexistence of different clinical entities and of *JAK2*V617F-positive and *JAK2*V617F-negative diseases in the same family is noteworthy.[131,134,135]

Diagnosis

Because of similarities with reactive forms characterized by an increased count of mature peripheral blood cells on one side, and the significant phenotypic overlapping among them on the other, diagnosis of different MPNs has traditionally been challenging; the availability of the new molecular markers is expected to facilitate diagnosis (Table 3). As a matter of fact, molecular genotyping is integral to the 2008 WHO diagnostic criteria,[136] and tests for *JAK2* or *MPL* mutation already have become a standard tool in the diagnostic work up of MPN (Fig. 2).[137] In fact, detection of one of these mutations unequivocally establishes by itself the presence of a clonal MPN and rules out the possibility of reactive erythrocytosis, thrombocytosis, or myelofibrosis. Unfortunately, they are of no help in distinguishing among the different forms of MPNs, although *JAK2* exon12

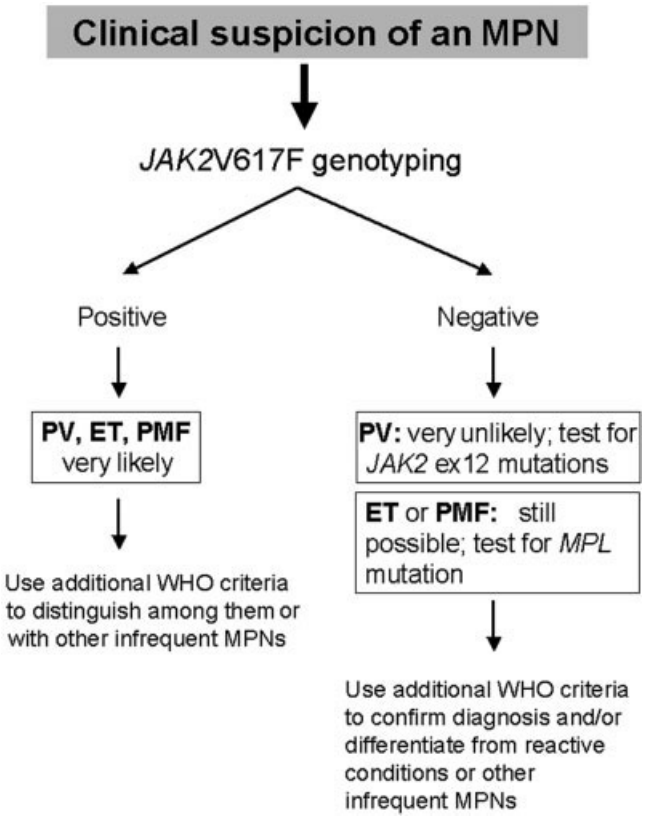

FIGURE 2. Rationale for using *JAK2*V617F genotyping in the diagnostic work-up of suspected MPN. See text for details.

mutations have not yet been reported outside PV, and no patient with PV has been found to harbor an *MPL* mutation.

In patients with evidence of increased red cell mass, according to WHO criteria,[136] demonstration of *JAK2*V617F mutation allows a diagnosis in greater than 95% of cases, as less than 2% of PV patients harbor *JAK2* exon 12 abnormalities.[16] It is debated whether a diagnosis of PV can still be tenable in the absence of *JAK2* mutation.[138,139]

The compelling criterion for a diagnosis of ET is a sustained platelet count of greater than 450×10^9/L. Notably, this value is lower than the one originally used by the 2001 WHO classification system (600×10^9/L),[5] because the latter might have led to inadvertently overlooking classic ET cases with a lower platelet count.[140] This assumption is supported by the discovery of the *JAK2*V617F mutation in some subjects who have a platelet count lower than 600×10^9/L.[47] Diagnosis of ET requires exclusion of reactive thrombocytosis[141,142] as well as of other MPNs that present with thrombocytosis. In particular, exclusion of CML with FISH or PCR analysis for *BCR-ABL* rearrangement is mandatory. Positivity for *JAK2*V617F or *MPL* mutation cumulatively account for 60% to 70% of ET cases. Therefore, the assessment of bone marrow morphology remains key to the diagnosis of ET; bone marrow cellularity is normal or slightly increased, with abundance of large, mature-appearing megakaryocytes devoid of morphological abnormalities and generally dispersed throughout the biopsy. This appearance is distinct from both the panmyelosis typical of PV or the predominant granulocytic hyperplasia with highly bizarre megakaryocytes, often found in abnormally tight clusters, with aberrant nuclear to cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei that are found in PMF, even in initial stages without overt fibrosis.[137,143]

Bone marrow histology is required for the diagnosis of PMF. Although advanced reticulin or collagenic fibrosis is typically associated with classic stages of PMF, some degree of reticulin fibrosis can be found as well as in PV, or more occasionally in ET. Therefore, fibrosis by itself is not synonym for PMF, and diagnosis of PMF can be made even in the absence of overt fibrosis.[8] Also the leukoerythroblastic features of blood smears, with immature myeloid precursors, nucleated red cells, and abnormally shaped erythrocytes (tear-drop cells), is very characteristic, but not diagnostic, of PMF. CML should be ruled out through *BCR-ABL* rearrangement analysis, while finding a positive *JAK2*V617F or *MPL* mutation allows exclusion of reactive forms of myelofibrosis (such as in infectious or inflammatory processes, metastatic cancer, and lymphoid disorders). Some cytogenetic abnormalities, such as del(13)(q12;q22), are frequently encountered and may be diagnostically specific in this context.[144] Anemia, palpable splenomegaly, and raised lactate dehydrogenase levels are additional diagnostic criteria.[8]

Clinical Course and Risk Stratification

Thrombosis, hemorrhage, evolution to post-polycythemic or post-thrombocythemic myelofibrosis, and AML transformation represent the most clinically relevant issues in the course of classic MPN.[145-147] Most thrombotic events occur at or in the two years before diagnosis.[148] However, epidemiologic inference from the European Collaboration on Low-dose Aspirin in Polycythemia (ECLAP) study[146,149] and the UK Medical Research Council Primary Thrombocythemia-1 (MRC PT-1) study[150] suggested that the cumulative rate of thrombosis during the disease course ranged from 2.5% to 5.0% and from 1.9% to 3% per patient-year in PV and ET, respectively, depending on whether the patient was in a low-risk or high-risk category.[146,150] In a large retrospective study of PV or ET patients who had suffered from a previous cardiovascular event, the calculated recurrence rate was 5.6% patient-year with a cumulative probability of 49.9% at 10 years.[151] Arterial thrombosis accounts for 60% to 70% of all cardiovascular events and includes acute myocardial infarction, ischemic stroke, and peripheral arterial occlusion. Events involving the venous system, more common among PV patients, are represented by lower extremity deep venous thrombosis, pulmonary embolism, and splanchnic vein thromboses (SVT, which includes portal vein thrombosis, mesenteric thrombosis, and thrombosis of the hepatic veins causing Budd-Chiari syndrome). The prevalence of SVT is unusually high among MPN patients[152]; however, diagnosis is often complicated by the hemodilution resulting from hypersplenism that makes blood cell counts unreliable, in particular as concerns evidence of increased red cell mass necessary for the diagnosis of PV.[153] Recent data indicate that at least 40% of patients with SVT

not attributable to other causes actually harbor the *JAK2*V617F mutation; therefore, *JAK2*V617F genotyping represents a first-line test for these conditions.[154,155] Occasional SVT patients harboring *MPL* mutation have also been reported.[156] Conversely, involvement of the microcirculatory system is more typically associated with ET and manifests as erythromelalgia (a rare disorder characterized by burning pain, warmth, and redness of the extremities due to arteriolar fibrosis and occlusion with platelet thrombi, typically aspirin-sensitive),[157] transient ischemic attacks, visual or hearing transitory defects, recurrent headache, and peripheral paresthesia; however, because of the lack of objective diagnostic criteria, true incidence of microvessel disturbances is difficult to assess.[158] Pathogenesis of thrombosis in classic MPNs is multifactorial; rheologic abnormalities due to increased red cell mass in PV, abnormal function of platelets and their enhanced interaction with leukocytes and endothelial cells, are all possible contributing factors[159]; however, neither thrombocytosis nor increased hematocrit (at least until 52%) are clearly associated with occurrence of thrombosis.[160]

Mortality rate is age-dependently increased in PV, being 1.6-fold and 3.3-fold higher than in the reference population in patients younger or older than 50 years, respectively.[161] Conversely, survival of ET patients is reduced by about 2-fold compared with the general population starting from the first decade after diagnosis.[162] Major causes of shortened survival in PV or ET are represented by thrombotic events and transformation to myelofibrosis or AML, which account for 41% and 13% of total deaths among 1,638 PV patients that were included in the observational arm of the ECLAP study.[146,163] An age of greater than 60 years and leukocytosis were incorporated in a predictive model for survival in ET that discriminated groups of patients with median survivals of 25, 17, and 10 years, respectively.[162] Therefore, because of the finding that thrombosis represents the most common event that complicates the courses of PV and ET, and eventually is the leading cause of death, it seems appropriate to use this clinical endpoint as the criterion for stratifying patients according to their risk.[164] Older age (greater than 60 years) and a previous history of thrombosis are standard risk factors for thrombosis in both PV and ET (Table 4), which have been validated in several studies.[146,148,165] In the presence of either of these, a patient is at high-risk,

TABLE 4. Risk-Stratification of Patients with Polycythemia Vera or Essential Thrombocythemia

RISK CATEGORY	AGE >60 YEARS OR HISTORY OF THROMBOSIS	GENERIC CARDIOVASCULAR RISK FACTORS
Low	No	No
Intermediate	No	Yes
High	Yes	Irrelevant

whereas when neither of these is present, the disease is low-risk. The role of generic cardiovascular risk factors, such as hypertension, diabetes, hyperlipidemia, smoking, or genetic alterations of hemostatic factors, is still controversial; however, patients who present with any of these abnormalities are prudentially considered to belong to an intermediate-risk category,[158,166] and both specific medical intervention and correction of life style issues, particularly smoking, should be aggressively pursued. Recent studies have demonstrated that leukocytosis is an additional independent risk factor for thrombosis,[162,167,168] particularly for acute myocardial infarction in PV. Furthermore, "low-risk" ET patients could be separated into two categories with a respective overall prevalence of thrombosis of 55% and 20% depending on the presence, or not, of an absolute leukocyte count greater than 8.7×10^9/L.[169] Finally, there is also evidence that *JAK2*V617F mutated status in ET,[47,170,171] and a high V617F allelic burden in both ET[47,172] and PV[172-173] are associated with increased risk of thrombosis. Therefore, both leukocytosis and *JAK2*V617F mutated status represent novel, powerful, disease-associated, risk factors; however, before they are included in current risk stratification criteria outlined in Table 4, they need validation in prospective studies.

Life expectancy in PMF is 31% lower than in an age-matched and sex-matched population, with a median survival of 5 years, although younger patients may experience longer survival.[161,174,175] Major causes of death are represented by the sequelae of portal hypertension or hepatic-splenoportal thrombosis, thromboses in various anatomic sites, heart failure due to splenic pooling, infections, pulmonary hypertension, bleeding caused by thrombocytopenia or hemostatic defects, and transformation to AML.[147] Prognostic staging systems for PMF have been developed that allow separation of patients with low-

TABLE 5. Prognostic Scoring Systems Used for Risk Assessment in Patients with PMF

PROGNOSTIC SCORING SYSTEM	PROGNOSTIC FACTORS	NO. OF PROGNOSTIC FACTORS BY RISK CATEGORY			NO. OF MONTHS OF SURVIVAL BY RISK CATEGORY		
		LOW	INTERMEDIATE	HIGH	LOW	INTERMEDIATE	HIGH
All patients							
Lille	Hb <10 g/dL WBC <4 or >30x10^9/L	0	1	2	93	26	13
Cervantes	Hb <10 g/dL PB Blasts ≥1% Constitutional symptoms	0-1	—	2-3	99	—	21
Mayo	Hb <10 g/dL WBC <4 or >30x10^9/L Plt <100x10^9/L Monocytes >1x10^9/L	0	1	≥2	173	61	26
Younger patients							
Cervantes, aged ≤55 y	Hb <10 g/dL PB Blasts >1% Constitut. symptoms	0-1	—	2-3	176	—	33
Dingli, aged <60 y	Hb <10 g/dL WBC <4 or >30x10^9/L Plt <100x10^9/L	0	1	2-3	155	69	24

Constitutional symptoms included unexplained fever, night sweats, or weight loss of greater than 10% of baseline value in the last 6 months.

PMF indicates primary myelofibrosis; Hb, hemoglobin; WBC, white blood cell count; PB Blasts, percentage of blasts in peripheral blood smears; Plt, platelet count.

risk and high-risk disease associated with significantly different survival times (Table 5). The most used "Lille score" includes anemia and abnormal leukocyte count as variables and effectively distinguishes patients with survival times that range from 1-8 years.[176] Stratification according to risk is of particular importance in younger patients who may potentially exploit the curative efficacy of allogeneic hematopoietic stem cell transplantation (HSCT). In this regard, "Cervantes"[175] and "Mayo" ad hoc scoring systems for patients aged younger than 55 years or 60 years have been developed[177] and represent useful instruments to aid both physician and patient to make the most appropriate therapeutic decision (Table 5). Presence of a *JAK2*V617F mutated state independently predicted leukemic transformation in a longitudinal prospective series of PMF patients,[178] whereas presence of *MPL*W5151L/K mutation was associated with more severe anemia.[60] However, because of conflicting results reported in similar studies,[179] these markers need further validation before being operationally incorporated into prognostic systems.

Transformation to post-polycythemic or post-thrombocytemic myelofibrosis represents the natural evolution of PV and ET, occurring late in the clinical course. The estimated rate is about 5% after 15 years from diagnosis of PV,[146] whereas data are scanty in ET. Criteria for the diagnosis of evolution to myelofibrosis have recently been proposed by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT; Table 6).[180] Survival is probably shortened by the development of myelofibrosis, and may be predicted by hemoglobin level and platelet and leukocyte counts according to a dynamic prognostic model recently developed in PV patients.[183]

Evolution to AML occurred in 1.3% of PV patients included in ECLAP study, at a median time of 8.4 years after diagnosis[163]; however, because of the short follow up, a precise estimate cannot be made, and information about ET is not yet available. Survival time is dismal, less than 6 months, although recipients of allogeneic HSCT may experience longer remission.[184] Advanced age, elevated leukocyte count, and longer disease duration were factors associated with increased risk of leukemic transformation.[163] An increased risk of AML was reported in patients who were treated with radioactive phosphorus or chlorambucil in the PVSG trial.[185] In addition, sequential or combined use of more

TABLE 6. Criteria for Establishing the Diagnosis of Evolution to Post-polycythemic or Post-thrombocythemic Myelofibrosis According to IWG-MRT Criteria[180]

CRITERIA FOR POST-POLYCYTHEMIC MYELOFIBROSIS
Required criteria
1. Documentation of a previous diagnosis of polycythemia vera as defined by WHO criteria[136]
2. Bone marrow fibrosis grade 2-3 (according to the European classification[181]) or grade 3-4 (according to standard classification[182])
Additional criteria
1. Anemia (below the reference range for appropriate age, sex, and altitude considerations) or sustained loss of either phlebotomy (in the absence of cytoreductive therapy) or cytoreductive treatment requirement for erythrocytosis
2. A leucoerythroblastic peripheral blood picture
3. Increasing splenomegaly of ≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly
4. Development of ≥1 of 3 constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)
CRITERIA FOR POST-THROMBOCYTHEMIC MYELOFIBROSIS
Required criteria
1. Documentation of a previous diagnosis of essential thrombocythemia as defined by WHO criteria[136]
2. Bone marrow fibrosis grade 2-3 (according to the European classification[181]) or grade 3-4 (according to standard classification[182])
Additional criteria
1. Anemia (below the reference range for appropriate age, sex, and altitude consideration) and a ≥20 g/L decrease from baseline hemoglobin level
2. A leucoerythroblastic peripheral blood picture.
3. Increasing splenomegaly of ≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly
4. Increased LDH (above reference level)
5. Development of ≥1 of 3 constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)

Diagnosis is made on the basis of meeting all required criteria plus two additional criteria.

WHO indicates World Health Organization; LDH, lactate dehydrogenase.

than one chemotherapeutic agent, including hydroxyurea (HU), significantly increased the rate of evolution to AML in PV patients in the observational arm of ECLAP study.[186]

Management of Classic MPN

Over the years, there has been a shortage of clinical studies specifically devoted to classic MPN. Most available information derives from a limited number of randomized clinical trials performed within national or international collaborative groups that include the Polycythemia Vera Study Group (PVSG),[165,186] the ECLAP study,[146,149] the "Bergamo trial,"[187] and the PT-1 trial[150]; however, the information they have provided represents the foundation for current treatment indications[189-192] as well as the basis for future studies (Table 7). Standardized criteria for assessing clinical and hematologic responses in PMF have been published[193,194] and will be of particular relevance for evaluation of novel molecularly target drugs. Conversely, similar criteria for patients with PV or ET are still lacking.

Cytoreductive Therapy in PV and ET

Treatment of patients with PV or ET should adhere to the standard risk stratification outlined above (Table 4). Phlebotomy is the cornerstone of treatment in low-risk patients with PV, aimed at reaching and maintaining a target hematocrit of less than 45% in men and less than 42% in women, according to standard recommendations.[165] The ultimate goal of this practice is to limit availability of iron to erythropoiesis, but often it will cause symptoms due to severe and prolonged iron deficiency; fatigue is being recognized as one major burden for quality of life in patients with PV.[127] In fact, there is wide variability in opinions and attitudes among US and non-US physicians concerning the optimal hematocrit target to be attained with phlebotomies.[195] Conversely, high-risk patients should receive myelosuppressive therapy, eventually in association with phlebotomy, and hydroxyurea (HU) is the drug of choice (Table 7). HU is an antimetabolite that prevents synthesis of DNA. It is also approved for the treatment of sickle cell anemia because of its capacity to reactivate synthesis of hemoglobin F, resulting in a significant decrease of occlusive and hemolytic events.[196] Superiority of HU compared with phlebotomy was suggested in a comparative analysis of the PVSG in the 1980s,[185] but no randomized trial to address this issue has yet been undertaken.

TABLE 7. Risk-Oriented Therapy in Polycythemia Vera (PV) and Essential Thrombocythemia (ET)

RISK CATEGORY	RISK FACTORS	PV	ET
Low	Age <60 y and no prior cardiovascular event	Phlebotomies plus low-dose aspirin	Nil, or low-dose aspirin (no consensus)
Intermediate	Generic cardiovascular risk factors		Low-dose aspirin (no consensus)
High	Age >60 y and/or prior cardiovascular event	Myelosuppression±Phlebotomies	Myelosuppression
		Low-dose aspirin	Low-dose aspirin

The use of low-dose aspirin in PV was exploited in the ECLAP study that randomized 518 low-risk patients in a double-blind, placebo-controlled trial.[149] The primary study endpoint (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, and major venous thromboembolism) was significantly lowered by aspirin (RR, 0.40; 95% confidence interval [CI] 0.18 to 0.91; $P = .02$), with only a small, nonsignificant increase of major hemorrhage (RR, 1.62; 95% CI, 0.27 to 9.71; $P = .60$); total and cardiovascular mortality were also reduced by 46% and 59%, respectively. Therefore, in the absence of a history of major bleeding, allergy to the drug or severe asthma, or gastric intolerance, low-dose aspirin (100 mg daily) is recommended regardless of risk category for all patients with PV.[149]

Low-risk patients with asymptomatic ET do not need therapy, although high-risk patients have the same indications for the use of HU as patients with PV.[197] In the "Bergamo trial," which randomized 114 high-risk patients to HU versus no treatment, the percentage of patients who developed thrombosis decreased from 24% to 3.6%.[188] HU was also superior to anagrelide, a nonmyelosuppressive platelet-lowering drug, in preventing arterial thrombosis in the randomized MRC-PT-1 trial, which included 809 high-risk patients, although venous thrombosis was reduced in the anagrelide arm.[54] Interestingly, *JAK2*V617F-positive patients had a better response and required lower doses of HU to control thrombocytosis compared with patients who did not have this mutation.[54] The target level at which the platelet count should be maintained with therapy in high-risk patients is currently set at 400-450 $\times$ 10^9/L, but this is not based on evidence.[197,198] Also, there is little rationale for the use of cytoreductive treatment to reduce extreme thrombocytosis (platelet count greater than 1,000 $\times$ 10^9/L) in an otherwise low-risk, asymptomatic patient.[199] Unlike PV, the safety and efficacy of low-dose aspirin use for ET has not formally been proven, but most patients in intermediate-risk or high-risk categories are currently advised to use the drug. Higher doses, up to 500 mg daily, may be required for acute symptoms because of microvascular disturbances, in particular erythromelalgia. Conversely, extreme thrombocytosis is considered a contraindication for aspirin use because of a possibly increased bleeding tendency due to acquired Von Willebrand disease.[158,200-202]

There has been some debate about potential leukemogenicity of HU, but although current evidence does not attribute to the drug a definite risk in this regard, it is appropriate to reserve HU use for patients at high risk of developing complications and in whom benefits expected from treatment overcome potential unwanted effects. Actually, transformation to AML is considered part of the natural history of MPN.[186]

Noncytotoxic Drugs for PV and ET

Interferon-alpha (IFN-α), a nonleukemogenic agent, has multiple potential activities against hematopoietic progenitor cell proliferation and differentiation which may justify its use in the youngest of patients with PV and ET. However, tolerance is often poor because of acute and chronic side effects that cause discontinuation of the drug in one-third of patients. IFN-α has been shown to effectively reduce the hematocrit or platelet count to a target level in the majority of cases,[203,204] and no thrombohemorragic events were recorded among 55 patients with PV who were followed for a median of 13 years.[205] Progressive decrease of *JAK2*V617F burden has been suggested in one study,[206] whereas changes were minimal in another study.[207] Notably, a recent, phase 2, multicenter study that used pegylated-IFN-α in 40 patients with PV reported complete molecular remission in 7 of these patients.[208] Finally, because IFN-α is not teratogenic and does not cross the placenta, it is recommended whenever there is the need for cytoreduction during pregnancy, according to current guidelines.[209]

Anagrelide has widely been used to control platelet count in patients with ET in all risk categories[210]; the

majority of patients achieved adequate control of thrombocytosis, although cardiovascular side effects (mainly palpitations and headache, less frequently congestive heart failure) may require early discontinuation of treatment.[211,212] The drug is considered devoid of any leukemogenic potential, but it should not be prescribed during pregnancy. On the basis of results of the PT-1 trial, anagrelide is not recommended in high-risk patients as an alternative to HU, and its rationale in otherwise low-risk or intermediate-risk patients should be carefully evaluated case by case.[213] However, according to recently published guidelines,[214] anagrelide could be successfully used in platelet count control in patients with PV or ET who are refractory and/or resistant, or who show poor tolerance, or who develop side effects to HU.

Management of PMF

The only approach that has resulted in a prolongation of survival time in PMF and has the potential to be curative is allogeneic HSCT.[215] At present, it should be reserved for patients with high-risk disease after careful clinical evaluation and thorough patient counseling, particularly considering the option of inclusion in trials with innovative drugs. Both myeloablative and reduced-intensity conditioning regimens have been used, with similar efficacy in terms of survival (3-year event-free survival in the range of 50% to 60%) but lower mortality rate with the use of the latter in older patients.[216] Therefore, a myeloablative strategy may be considered as the most appropriate for younger patients, whereas the reduced-intensity regimen would be the best for older patients. Furthermore, in patients who relapse after HSCT, a graft-versus-myelofibrosis effect could be demonstrated after donor-lymphocyte infusion with a remarkable reduction of bone marrow fibrosis.[217,218] Factors that have been reported as having a favorable impact on overall survival after HSCT include a conditioning regimen with busulfan/cyclophosphamide, younger age, high platelet count, low comorbidity index, low risk according to the Dupriez score, normal karyotype, hemoglobin of greater than 100 g/L, absence of circulating blasts, and absence of osteosclerosis.[219-221] The usefulness of pretransplant splenectomy still remains controversial.[215,222,223]

Given that a conventional drug therapy does not significantly modify disease course and is largely ineffective, it is reserved for patients who present either with symptomatic anemia or splenomegaly. Androgens,[224] prednisone,[224] erythropoiesis-stimulating agents,[225-227] and danazol[228,229] are all variably used with measurable effect in a few patients. Low-dose thalidomide in combination with prednisone improves anemia or thrombocytopenia in 30% to 50% of cases.[230-234] Lenalidomide, a thalidomide analog, has produced excellent and durable responses in the relatively infrequent PMF patients who have the del(5q) abnormality,[235] and it can be recommended as first-line therapy in this patient subset. When there is the need to control excessive myeloproliferation, ie, leukocytosis, thrombocytosis, or progressive splenomegaly, HU is the current drug of choice.[236] Several other drugs, including busulfan,[237] melphalan,[238] and 2-chlorodeoxyadenosine,[239] have been used particularly in HU-refractory patients, but results are generally dismal. Splenectomy has a role for alleviating mechanical symptoms due to extreme splenomegaly and can also ameliorate anemia in approximately 25% of transfusion-dependent patients.[240] However, splenectomy in PMF bears an approximately 10% procedure-related mortality, and it should be performed by experienced surgeons. Furthermore, up to 25% of patients present with accelerated hepatomegaly and extreme thrombocytosis after splenectomy, and these patients require further cytoreduction.[240,241] Splenic irradiation is reserved for patients who cannot undergo splenectomy for any reason, but the efficacy of this therapy is poor, and subsequent cytopenias are often severe. Conversely, radiation therapy has a defined role in the treatment of nonhepatosplenic extramedullary hematopoiesis, such as in cases of spinal cord compression by foci of eterotopic hematopoiesis.[242-245]

Prospect for Molecularly Targeted Therapy in Classic MPN

The involvement of JAK-STAT pathways in most patients who have classic MPN and harbor mutations in *JAK2* or *MPL* and the experimental evidence that suggests that the same signaling abnormalities may be at the basis of mutation-negative patients[20] are behind active efforts to develop anti-JAK2 drugs. Many molecules have undergone preclinical testing, in vitro and also in vivo, and some have already been introduced into clinical trials.[246-252] A very incomplete list of molecules that may or may not have selective anti-JAK2 activity is reported in Table 8. Concerning selective JAK2 inhibitors, we have listed only those that are already in clinical trials or whose activity has been demonstrated in

TABLE 8. Innovative Therapies for Classic MPN

DRUG	MAIN TARGETS	IN CLINICAL TRIAL
JAK2 selective inhibitors		
INCB018424	JAK2	Yes
XL019	JAK2	Yes
TG101348	JAK2	Yes
Non-JAK2 selective inhibitors		
CEP-701 (Leustartinib)	FLT3	Yes
MK-0457	Aurora Kinase, FLT3, BCR-ABL	Yes
Erlotinib	EGFR	Yes
ITF2357	Histone deacetylases	Yes
Tipifarnib	FT	Yes

MPN indicates myeloproliferative neoplasm; FLT3, FMS-like tyrosine kinase 3; EGFR, epidermal growth factor receptor; FT, farnesyl transferase.

*JAK2*V617F-mutated murine models (TG101348).[248] Among these, INCB018424, XL019, CEP-701, and TG101348 are currently undergoing clinical trials in patients with advanced stages of PMF, post-PV/ET myelofibrosis, PV, and *JAK2*V617F-positive ET.[253-256] Preliminary results have been encouraging in terms of activity against splenomegaly and constitutional symptoms,[253] with minimal toxicity. Although the number of patients treated until now is less than 100 with any single drug and, thus, prevents us from making any definitive comment, the hope that this molecularly targeted approach may finally result in improving quality of life and possibly the chance of cure for patients with classic MPN is enormous.

Patient Resources

During the last few years, we have witnessed a renewed interest in the MPN field among scientific communities and pharmaceutical companies; at the same time, the patient community is growing in awareness and strength. There are several focused resources for patient information and support that include the Myeloproliferative Disorders Research Consortium (MPD-RC, an international research consortium funded by the National Cancer Institute; http://www.mpd-rc.org), the Myeloproliferative Disorders Foundation (committed to promoting focused research and international expert cooperation and also devoted to patient education and support; http://www.mpdinfo.org), the Mastocytosis Society (http://www.tmsforacure.org), and several online support groups (such as http://www.acor.org; http://www.mpdsupport.org). Among non-US resources are the Myeloproliferative Disorders Australia (MPD-Oz; http://www.mpd-oz.org), the Italian Mielofibrosi Insieme (for patients with PMF; http://www.myelofibrosis.net), and the Gruppo Italiano per le Malattie Ematologiche Maligne dell'Adulto-GIMEMA (http://www.gimema.org). ■

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