Advances in understanding and management of myeloproliferative neoplasms

Advances in Understanding and Management of Myeloproliferative Neoplasms

Alessandro M. Vannucchi, Paola Guglielmelli and Ayalew Tefferi

CA Cancer J Clin 2009;59;171-191; originally published online Apr 15, 2009;
DOI: 10.3322/caac.20009

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.**

CME CNE

To earn free CME credit or nursing contact hours for successfully completing the online quiz based on this article, go to **http://CME.AmCancerSoc.org.**

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

[1]Associate Professor of Hematology, Department of Hematology, University of Florence, Florence, Italy. [2]Research Fellow at the Department of Hematology, University of Florence, Florence, Italy. [3]Professor of Medicine and Hematology, Mayo Clinic College of Medicine, Rochester, NY.

Corresponding authors: Alessandro M. Vannucchi, MD, Hematology Unit, Dip. Area Critica, University of Florence, Viale Morgagni 85, 50134 Florence, Italy; amvannucchi@unifi.it and Ayalew Tefferi, MD, Division of Hematology, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905; tefferi.ayalew@mayo.edu

DISCLOSURES: This study was supported by Associazione Italiana per la Ricerca sul Cancro, Milano; Istituto Toscano Tumori; MIUR (COFIN 2006067001_003). The authors report no conflicts of interest.

 doi:10.3322/caac.20009.

Available online at http://cajournal.org and http://cacancerjournal.org

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-

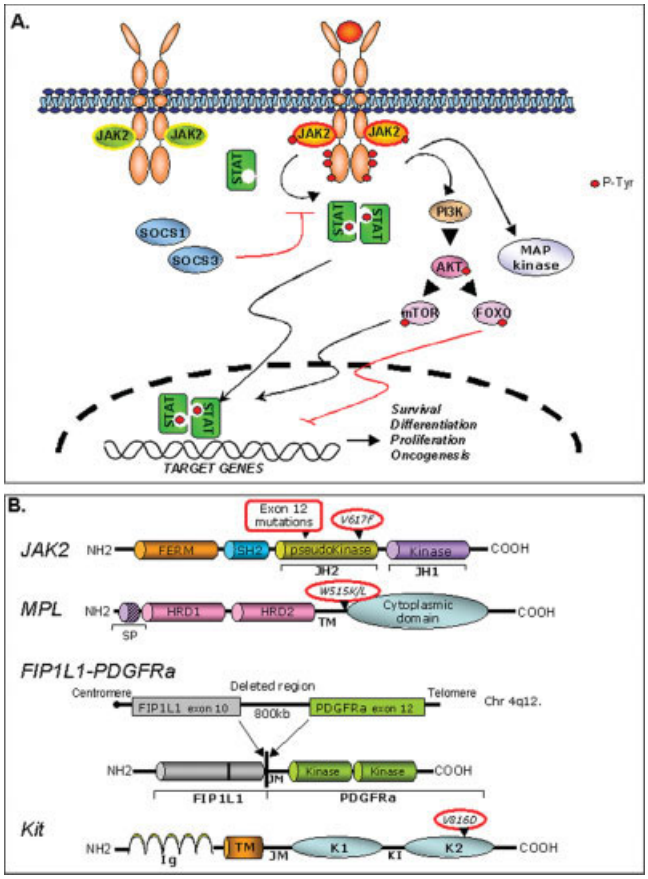

FIGURE 1. **(A)** In normal hematopoietic cells, signaling is initiated when cytokines bind to and activate their cell surface type-1 receptors, which have molecules of JAK2 associated to the cytoplasmic domains. After ligand engagement (the pathway activated by EPO bound to the EPOR is herein schematized) 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

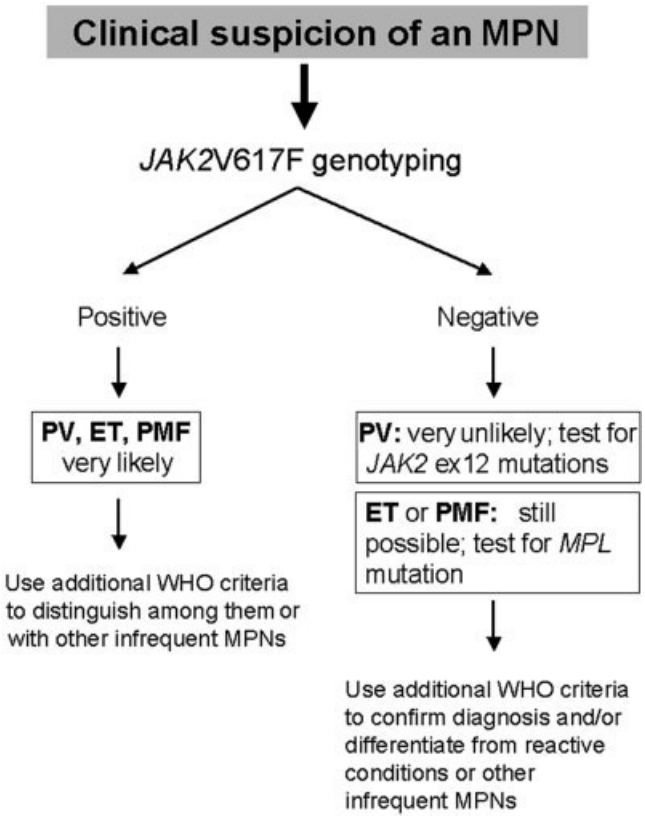

FIGURE 2. Rationale for using *JAK2*V617F genotyping in the diagnostic work-up of suspected MPN. See text for details.

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). ■

REFERENCES

1. Dameshek W. Some speculations on the myeloproliferative syndromes. Blood 1951; 6:372–375.
2. Mesa RA, Verstovsek S, Cervantes F, et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): Consensus on terminology by the international working group for myelofibrosis research and treatment (IWG-MRT). Leuk Res 2007;31:737–740.
3. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst 1960;25: 85–109.
4. Tefferi A. The history of myeloproliferative disorders: before and after Dameshek. Leukemia 2008;22:3–13.
5. Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001.
6. Spivak JL. Diagnosis of the myeloproliferative disorders: resolving phenotypic mimicry. Semin Hematol 2003;40:1–5.
7. Kaushansky K. The chronic myeloproliferative disorders and mutation of JAK2: Dameshek's 54 year old speculation comes of age. Best Pract Res Clin Haematol 2007;20:5–12.
8. Tefferi A, Thiele J, Orazi A, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood 2007;110:1092–1097.
9. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973;243:290–293.
10. Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 2003;348:1201–1214.
11. Nagata H, Worobec AS, Oh CK, et al. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. Proc Natl Acad Sci U S A 1995;92:10560–10564.
12. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005;7: 387–397.
13. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signaling causes polycythaemia vera. Nature 2005;434:1144–1148.
14. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779–1790.
15. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054–1061.

16. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 2007;356:459–468.
17. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med 2006;3:e270.
18. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 2006;108:3472–3476.
19. Cazzola M, Skoda R. Gain of function, loss of control—a molecular basis for chronic myeloproliferative disorders. Haematologica 2005;90:871–874.
20. Levine RL, Pardanani A, Tefferi A, Gilliland DG. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. Nat Rev Cancer 2007;7:673–683.
21. Delhommeau F, Pisani DF, James C, Casadevall N, Constantinescu S, Vainchenker W. Oncogenic mechanisms in myeloproliferative disorders. Cell Mol Life Sci 2006;63:2939–2953.
22. Tefferi A, Gilliland DG. Oncogenes in myeloproliferative disorders. Cell Cycle 2007;6:550–566.
23. Saharinen P, Silvennoinen O. The pseudokinase domain is required for suppression of basal activity of Jak2 and Jak3 tyrosine kinases and for cytokine-inducible activation of signal transduction. J Biol Chem 2002;277:47954–47963.
24. Feener EP, Rosario F, Dunn SL, Stancheva Z, Myers MG, Jr. Tyrosine phosphorylation of Jak2 in the JH2 domain inhibits cytokine signaling. Mol Cell Biol 2004;24:4968–4978.
25. Mertens C, Darnell JE, Jr. SnapShot: JAK-STAT signaling. Cell 2007;131:612.
26. Starr R, Hilton DJ. Negative regulation of the JAK/STAT pathway. Bioessays 1999; 21:47–52.
27. Shuai K, Liu B. Regulation of JAK-STAT signaling in the immune system. Nat Rev Immunol 2003;3:900–911.
28. Sasaki A, Yasukawa H, Shouda T, Kitamura T, Dikic I, Yoshimura A. CIS3/SOCS-3 suppresses erythropoietin (EPO) signaling by binding the EPO receptor and JAK2. J Biol Chem 2000;275:29338–29347.
29. Stofega MR, Herrington J, Billestrup N, Carter-Su C. Mutation of the SHP-2 binding site in growth hormone (GH) receptor prolongs GH-promoted tyrosyl phosphorylation of GH receptor, JAK2, and STAT5B. Mol Endocrinol 2000;14:1338–1350.
30. Huang LJ, Constantinescu SN, Lodish HF. The N-terminal domain of Janus kinase 2 is required for Golgi processing and cell surface expression of erythropoietin receptor. Mol Cell 2001;8:1327–1338.
31. Royer Y, Staerk J, Costuleanu M, Courtoy PJ, Constantinescu SN. Janus kinases affect thrombopoietin receptor cell surface localization and stability. J Biol Chem 2005;280:27251–27261.
32. Lu X, Levine R, Tong W, et al. Expression of a homodimeric type I cytokine receptor is required for JAK2V617F-mediated transformation. Proc Natl Acad Sci U S A 2005;102:18962–18967.
33. Garcon L, Rivat C, James C, et al. Constitutive activation of STAT5 and Bcl-xL overexpression can induce endogenous erythroid colony formation in human primary cells. Blood 2006;108:1551–1554.
34. Prchal JF, Axelrad AA. Letter: Bone-marrow responses in polycythemia vera. N Engl J Med 1974;290:1382.
35. Wernig G, Mercher T, Okabe R, Levine RL, Lee BH, Gilliland DG. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood 2006;107:4274–4281.
36. Lacout C, Pisani DF, Tulliez M, Gachelin FM, Vainchenker W, Villeval JL. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. Blood 2006;108:1652–1660.
37. Bumm TG, Elsea C, Corbin AS, et al. Characterization of murine JAK2V617F-positive myeloproliferative disease. Cancer Res 2006;66:11156–11165.
38. Zaleskas VM, Krause DS, Lazarides K, et al. Molecular pathogenesis and therapy of polycythemia induced in mice by JAK2 V617F. PLoS ONE 2006;1:e18.
39. Tiedt R, Hao-Shen H, Sobas MA, et al. Ratio of mutant JAK2–V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. Blood 2008;111:3931–3940.
40. Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. Leukemia 2008; 22: 1299–1307.
41. Szpurka H, Tiu R, Murugesan G, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood 2006;108:2173–2181.
42. Steensma DP, McClure RF, Karp JE, et al. JAK2 V617F is a rare finding in de novo acute myeloid leukemia, but STAT3 activation is common and remains unexplained. Leukemia 2006;20:971–978.
43. Renneville A, Quesnel B, Charpentier A, et al. High occurrence of JAK2 V617 mutation in refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Leukemia 2006;20:2067–2070.
44. Nishii K, Nanbu R, Lorenzo VF, et al. Expression of the JAK2 V617F mutation is not found in de novo AML and MDS but is detected in MDS-derived leukemia of megakaryoblastic nature. Leukemia 2007; 21:1337–1338.
45. Vizmanos JL, Ormazabal C, Larrayoz MJ, Cross NC, Calasanz MJ. JAK2 V617F mutation in classic chronic myeloproliferative diseases: a report on a series of 349 patients. Leukemia 2006;20:534–535.
46. Passamonti F, Rumi E, Pietra D, et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood 2006;107:3676–3682.
47. Antonioli E, Guglielmelli P, Poli G, et al. Influence of JAK2V617F allele burden on phenotype in essential thrombocythemia. Haematologica 2008;93:41–48.
48. Pardanani A, Fridley BL, Lasho TL, Gilliland DG, Tefferi A. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. Blood 2008; 111:2785–2789.
49. Skoda R. The genetic basis of myeloproliferative disorders. Hematology Am Soc Hematol Educ Program 2007;2007:1–10.
50. Nussenzveig RH, Swierczek SI, Jelinek J, et al. Polycythemia vera is not initiated by JAK2V617F mutation. Exp Hematol 2007; 35:32–38.
51. Theocharides A, Boissinot M, Girodon F, et al. Leukemic blasts in transformed JAK2–V617F-positive myeloproliferative disorders are frequently negative for the JAK2–V617F mutation. Blood 2007;110: 375–379.
52. Campbell PJ, Baxter EJ, Beer PA, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood 2006;108:3548–3555.
53. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia 2007;21:1960–1963.
54. Campbell PJ, Scott LM, Buck G, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet 2005; 366:1945–1953.
55. Pietra D, Li S, Brisci A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood 2007;111:1686–1689.
56. Pardanani A, Lasho T, Finke CM, et al. Extending JAK2V617F and MPL515 mutation analysis to single myeloid colonies and T and B lymphocytes. Stem Cells 2007;25:2358–2362.
57. Chaligne R, James C, Tonetti C, et al. Evidence for MPL W515L/K mutations in hematopoietic stem cells in primitive myelofibrosis. Blood 2007;110:3735–3743.
58. Pardanani A, Lasho TL, Finke C, Markovic SN, Tefferi A. Demonstration of MPLW515K, but not JAK2V617F, in in vitro expanded CD4+ T lymphocytes. Leukemia 2007;21:2206–2207.
59. Kaushansky K. The molecular mechanisms that control thrombopoiesis. J Clin Invest 2005;115:3339–3347.
60. Guglielmelli P, Pancrazzi A, Bergamaschi G, et al. Anaemia characterises patients with myelofibrosis harbouring Mpl mutation. Br J Haematol 2007;137:244–247.
61. Beer PA, Campbell P, Scott LM, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood 2008; 112:141–149.
62. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. Blood 2008; 112:844–847.
63. Williams DM, Kim AH, Rogers O, Spivak JL, Moliterno AR. Phenotypic variations and new mutations in JAK2 V617F-negative polycythemia vera, erythrocytosis, and idiopathic myelofibrosis. Exp Hematol 2007;35:1641–1646.
64. Chaligne R, Tonetti C, Besancenot R, et al. New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G(1)/S-phase transition. Leukemia 2008;22:1557–1566.
65. Lasho TL, Pardanani A, McClure RF, et al. Concurrent MPL515 and JAK2V617F mutations in myelofibrosis: chronology of clonal emergence and changes in mutant allele burden over time. Br J Haematol 2006;135:683–687.

66. Fletcher S, Bain B. Diagnosis and treatment of hypereosinophilic syndromes. Curr Opin Hematol 2007;14:37–42.
67. Buitenhuis M, Verhagen LP, Cools J, Coffer PJ. Molecular mechanisms underlying FIP1L1-PDGFRA-mediated myeloproliferation. Cancer Res 2007;67:3759–3766.
68. Cools J, Stover EH, Boulton CL, et al. PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFRalpha-induced myeloproliferative disease. Cancer Cell 2003;3:459–469.
69. Robyn J, Lemery S, McCoy JP, et al. Multilineage involvement of the fusion gene in patients with FIP1L1/PDGFRA-positive hypereosinophilic syndrome. Br J Haematol 2006;132:286–292.
70. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 1994;77:307–316.
71. Wilkinson K, Velloso ER, Lopes LF, et al. Cloning of the t(1;5)(q23;q33) in a myeloproliferative disorder associated with eosinophilia: involvement of PDGFRB and response to imatinib. Blood 2003;102:4187–4190.
72. Wang LC, Swat W, Fujiwara Y, et al. The TEL/ETV6 gene is required specifically for hematopoiesis in the bone marrow. Genes Dev 1998;12:2392–2402.
73. Carroll M, Tomasson MH, Barker GF, Golub TR, Gilliland DG. The TEL/platelet-derived growth factor beta receptor (PDGF beta R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF beta R kinase-dependent signaling pathways. Proc Natl Acad Sci U S A 1996;93:14845–14850.
74. Valent P. Systemic mastocytosis. Cancer Treat Res 2008;142:399–419.
75. Cairoli R, Beghini A, Grillo G, et al. Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. Blood 2006;107:3463–3468.
76. Sihto H, Sarlomo-Rikala M, Tynninen O, et al. KIT and platelet-derived growth factor receptor alpha tyrosine kinase gene mutations and KIT amplifications in human solid tumors. J Clin Oncol 2005;23:49–57.
77. Harir N, Boudot C, Friedbichler K, et al. Oncogenic Kit controls neoplastic mast cell growth through a Stat5/PI3-kinase signaling cascade. Blood 2008;112:2463–2473.
78. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292–2302.
79. Gilliland DG, Blanchard KL, Levy J, Perrin S, Bunn HF. Clonality in myeloproliferative disorders: analysis by means of the polymerase chain reaction. Proc Natl Acad Sci U S A 1991;88:6848–6852.
80. Fialkow PJ, Gartler SM, Yoshida A. Clonal origin of chronic myelocytic leukemia in man. Proc Natl Acad Sci U S A 1967;58:1468–1471.
81. Barr RD, Fialkow PJ. Clonal origin of chronic myelocytic leukemia. N Engl J Med 1973;289:307–309.
82. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem-cell and probable clonal origin of the disease. N Engl J Med 1976;295:913–916.
83. Fialkow PJ, Jacobson RJ, Papayannopoulou T. Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am J Med 1977;63:125–130.
84. Fialkow PJ, Denman AM, Jacobson RJ, Lowenthal MN. Chronic myelocytic leukemia. Origin of some lymphocytes from leukemic stem cells. J Clin Invest 1978;62:815–823.
85. Jacobson RJ, Salo A, Fialkow PJ. Agnogenic myeloid metaplasia: a clonal proliferation of hematopoietic stem cells with secondary myelofibrosis. Blood 1978;51:189–194.
86. Martin PJ, Najfeld V, Hansen JA, Penfold GK, Jacobson RJ, Fialkow PJ. Involvement of the B-lymphoid system in chronic myelogenous leukaemia. Nature 1980;287:49–50.
87. Fialkow PJ, Faguet GB, Jacobson RJ, Vaidya K, Murphy S. Evidence that essential thrombocythemia is a clonal disorder with origin in a multipotent stem cell. Blood 1981;58:916–919.
88. Reeder TL, Bailey RJ, Dewald GW, Tefferi A. Both B and T lymphocytes may be clonally involved in myelofibrosis with myeloid metaplasia. Blood 2003;101:1981–1983.
89. Delhommeau F, Dupont S, Tonetti C, et al. Evidence that the JAK2 G1849T (V617F) mutation occurs in a lymphomyeloid progenitor in polycythemia vera and idiopathic myelofibrosis. Blood 2007;109:71–77.
90. Tefferi A, Gangat N, Wolanskyj AP, et al. 20+ yr without leukemic or fibrotic transformation in essential thrombocythemia or polycythemia vera: predictors at diagnosis. Eur J Haematol 2008;80:386–390.
91. Tefferi A, Elliott MA, Pardanani A. Atypical myeloproliferative disorders: diagnosis and management. Mayo Clin Proc 2006;81:553–563.
92. Hehlmann R, Hochhaus A, Baccarani M. Chronic myeloid leukaemia. Lancet 2007;370:342–350.
93. Schiffer CA. BCR-ABL tyrosine kinase inhibitors for chronic myelogenous leukemia. N Engl J Med 2007;357:258–265.
94. Kantarjian H, Schiffer C, Jones D, Cortes J. Monitoring the response and course of chronic myeloid leukemia in the modern era of BCR-ABL tyrosine kinase inhibitors: practical advice on the use and interpretation of monitoring methods. Blood 2008;111:1774–1780.
95. Elliott MA, Hanson CA, Dewald GW, Smoley SA, Lasho TL, Tefferi A. WHO-defined chronic neutrophilic leukemia: a long-term analysis of 12 cases and a critical review of the literature. Leukemia 2005;19:313–317.
96. Elliott MA. Chronic neutrophilic leukemia and chronic myelomonocytic leukemia: WHO defined. Best Pract Res Clin Haematol 2006;19:571–593.
97. Gotlib J. Chronic eosinophilic leukemia/hypereosinophilic syndrome. Cancer Treat Res 2008;142:69–106.
98. Tefferi A, Patnaik MM, Pardanani A. Eosinophilia: secondary, clonal and idiopathic. Br J Haematol 2006;133:468–492.
99. Simon HU, Plotz SG, Dummer R, Blaser K. Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. N Engl J Med 1999;341:1112–1120.
100. Gotlib J, Cross NC, Gilliland DG. Eosinophilic disorders: molecular pathogenesis, new classification, and modern therapy. Best Pract Res Clin Haematol 2006;19:535–569.
101. Kalac M, Quintas-Cardama A, Vrhovac R, Kantarjian H, Verstovsek S. A critical appraisal of conventional and investigational drug therapy in patients with hypereosinophilic syndrome and clonal eosinophilia. Cancer 2007;110:955–964.
102. Garrett JK, Jameson SC, Thomson B, et al. Anti-interleukin-5 (mepolizumab) therapy for hypereosinophilic syndromes. J Allergy Clin Immunol 2004;113:115–119.
103. Sefcick A, Sowter D, DasGupta E, Russell NH, Byrne JL. Alemtuzumab therapy for refractory idiopathic hypereosinophilic syndrome. Br J Haematol 2004;124:558–559.
104. Garcia-Montero AC, Jara-Acevedo M, Teodosio C, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. Blood 2006;108:2366–2372.
105. Patnaik MM, Rindos M, Kouides PA, Tefferi A, Pardanani A. Systemic mastocytosis: a concise clinical and laboratory review. Arch Pathol Lab Med 2007;131:784–791.
106. Pardanani A, Elliott M, Reeder T, et al. Imatinib for systemic mast-cell disease. Lancet 2003;362:535–536.
107. Tefferi A, Verstovsek S, Pardanani A. How we diagnose and treat WHO-defined systemic mastocytosis in adults. Haematologica 2008;93:6–9.
108. Orazi A, Germing U. The myelodysplastic/myeloproliferative neoplasms: myeloproliferative diseases with dysplastic features. Leukemia 2008;22:1308–1319.
109. Hirsch-Ginsberg C, LeMaistre AC, Kantarjian H, et al. RAS mutations are rare events in Philadelphia chromosome-negative/bcr gene rearrangement-negative chronic myelogenous leukemia, but are prevalent in chronic myelomonocytic leukemia. Blood 1990;76:1214–1219.
110. Tartaglia M, Niemeyer CM, Fragale A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 2003;34:148–150.
111. Levine RL, Loriaux M, Huntly BJ, et al. The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. Blood 2005;106:3377–3379.
112. Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol 2002;20:2429–2440.
113. Aribi A, Borthakur G, Ravandi F, et al. Activity of decitabine, a hypomethylating agent, in chronic myelomonocytic leukemia. Cancer 2007;109:713–717.
114. Kantarjian H, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer 2006;106:1794–1803.
115. Kantarjian H, Oki Y, Garcia-Manero G, et al. Results of a randomized study of 3 schedules of low-dose decitabine in higher-

risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood 2007;109:52–57.

116. Fend F, Horn T, Koch I, Vela T, Orazi A. Atypical chronic myeloid leukemia as defined in the WHO classification is a JAK2 V617F negative neoplasm. Leuk Res 2008;32:1931–1935.
117. Shaw GR. Ringed sideroblasts with thrombocytosis: an uncommon mixed myelodysplastic/myeloproliferative disease of older adults. Br J Haematol 2005;131:180–184.
118. Schmitt-Graeff AH, Teo SS, Olschewski M, et al. JAK2V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica 2008;93:34–40.
119. Steensma DP, Caudill JS, Pardanani A, McClure RF, Lasho TL, Tefferi A. MPL W515 and JAK2 V617 mutation analysis in patients with refractory anemia with ringed sideroblasts and an elevated platelet count. Haematologica 2006;91:ECR57.
120. Atallah E, Nussenzveig R, Yin CC, et al. Prognostic interaction between thrombocytosis and JAK2 V617F mutation in the WHO subcategories of myelodysplastic/myeloproliferative disease-unclassifiable and refractory anemia with ringed sideroblasts and marked thrombocytosis. Leukemia 2008;22:1295–1298.
121. Gotlib J. Molecular classification and pathogenesis of eosinophilic disorders: 2005 update. Acta Haematol 2005;114:7–25.
122. Tefferi A, Lasho TL, Brockman SR, Elliott MA, Dispenzieri A, Pardanani A. FIP1L1-PDGFRA and c-kit D816V mutation-based clonality studies in systemic mast cell disease associated with eosinophilia. Haematologica 2004;89:871–873.
123. Florian S, Esterbauer H, Binder T, et al. Systemic mastocytosis (SM) associated with chronic eosinophilic leukemia (SM-CEL): detection of FIP1L1/PDGFRalpha, classification by WHO criteria, and response to therapy with imatinib. Leuk Res 2006;30:1201–1205.
124. Baccarani M, Cilloni D, Rondoni M, et al. The efficacy of imatinib mesylate in patients with FIP1L1-PDGFRalpha-positive hypereosinophilic syndrome. Results of a multicenter prospective study. Haematologica 2007;92:1173–1179.
125. Macdonald D, Aguiar RC, Mason PJ, Goldman JM, Cross NC. A new myeloproliferative disorder associated with chromosomal translocations involving 8p11: a review. Leukemia 1995;9:1628–1630.
126. Macdonald D, Reiter A, Cross NC. The 8p11 myeloproliferative syndrome: a distinct clinical entity caused by constitutive activation of FGFR1. Acta Haematol 2002;107:101–107.
127. Mesa RA, Niblack J, Wadleigh M, et al. The burden of fatigue and quality of life in myeloproliferative disorders (MPDs): an international Internet-based survey of 1179 MPD patients. Cancer 2007;109:68–76.
128. Teofili L, Giona F, Martini M, et al. The revised WHO diagnostic criteria for Ph-negative myeloproliferative diseases are not appropriate for the diagnostic screening of childhood polycythemia vera and essential thrombocythemia. Blood 2007;110:3384–3386.
129. Randi ML, Putti MC, Scapin M, et al. Pediatric patients with essential thrombocythemia are mostly polyclonal and V617FJAK2 negative. Blood 2006;108:3600–3602.
130. Rollison DE, Howlader N, Smith MT, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001–2004, using data from the NAACCR and SEER programs. Blood 2008;112:45–52.
131. Bellanne-Chantelot C, Chaumarel I, Labopin M, et al. Genetic and clinical implications of the Val617Phe JAK2 mutation in 72 families with myeloproliferative disorders. Blood 2006;108:346–352.
132. Kralovics R, Stockton DW, Prchal JT. Clonal hematopoiesis in familial polycythemia vera suggests the involvement of multiple mutational events in the early pathogenesis of the disease. Blood 2003;102:3793–3796.
133. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Bjorkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24577 first-degree relatives of 11039 patients with myeloproliferative neoplasms in Sweden. Blood 2008;112:2199–2204.
134. Rumi E, Passamonti F, Della Porta MG, et al. Familial chronic myeloproliferative disorders: clinical phenotype and evidence of disease anticipation. J Clin Oncol 2007;25:5630–5635.
135. Rumi E, Passamonti F, Pietra D, et al. JAK2 (V617F) as an acquired somatic mutation and a secondary genetic event associated with disease progression in familial myeloproliferative disorders. Cancer 2006;107:2206–2211.
136. Swerdlow SH, Campo E, Harris NL, et al. (eds.). WHO classification of tumors of haematopoietic and lymphoid tissues. 4th Edition. IARC, Lyon. 2008.
137. Tefferi A, Vardiman JW. The diagnostic interface between histology and molecular tests in myeloproliferative disorders. Curr Opin Hematol 2007;14:115–122.
138. Wang YL, Vandris K, Jones A, et al. JAK2 Mutations are present in all cases of polycythemia vera. Leukemia 2008;22:1289.
139. Spivak JL, Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis and primary myelofibrosis: an alternative proposal. Blood 2008;112:231–239.
140. Lengfelder E, Hochhaus A, Kronawitter U, et al. Should a platelet limit of 600 × 10(9)/l be used as a diagnostic criterion in essential thrombocythaemia? An analysis of the natural course including early stages. Br J Haematol 1998;100:15–23.
141. Schafer AI. Thrombocytosis. N Engl J Med 2004;350:1211–1219.
142. Vannucchi AM, Barbui T. Thrombocytosis and thrombosis. Hematology Am Soc Hematol Educ Program 2007;2007:363–370.
143. Thiele J, Kvasnicka HM, Vardiman J. Bone marrow histopathology in the diagnosis of chronic myeloproliferative disorders: a forgotten pearl. Best Pract Res Clin Haematol 2006;19:413–437.
144. Tefferi A, Mesa RA, Schroeder G, Hanson CA, Li CY, Dewald GW. Cytogenetic findings and their clinical relevance in myelofibrosis with myeloid metaplasia. Br J Haematol 2001;113:763–771.
145. Harrison CN, Green AR. Essential thrombocythaemia. Best Pract Res Clin Haematol 2006;19:439–453.
146. Marchioli R, Finazzi G, Landolfi R, et al. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. J Clin Oncol 2005;23:2224–2232.
147. Tefferi A. Primary myelofibrosis. Cancer Treat Res 2008;142:29–49.
148. Policitemia GIS. Polycythemia vera: the natural history of 1213 patients followed for 20 years. Gruppo Italiano Studio Policitemia. Ann Intern Med 1995;123:656–664.
149. Landolfi R, Marchioli R, Kutti J, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. N Engl J Med 2004;350:114–124.
150. Harrison CN, Campbell PJ, Buck G, et al. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. N Engl J Med 2005;353:33–45.
151. De Stefano V, Za T, Rossi E, et al. Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. Haematologica 2008;93:208–218.
152. Briere JB. Budd-Chiari syndrome and portal vein thrombosis associated with myeloproliferative disorders: diagnosis and management. Semin Thromb Hemost 2006;32:208–218.
153. Chait Y, Condat B, Cazals-Hatem D, et al. Relevance of the criteria commonly used to diagnose myeloproliferative disorder in patients with splanchnic vein thrombosis. Br J Haematol 2005;129:553–560.
154. Kiladjian JJ, Cervantes F, Leebeek FW, et al. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. Blood 2008;111:4922–4929.
155. Primignani M, Barosi G, Bergamaschi G, et al. Role of the JAK2 mutation in the diagnosis of chronic myeloproliferative disorders in splanchnic vein thrombosis. Hepatology 2006;44:1528–1534.
156. Bergamaschi GM, Primignani M, Barosi G, et al. MPL and JAK2 exon 12 mutations in patients with the Budd-Chiari syndrome or extrahepatic portal vein obstruction. Blood 2008;111:4418.
157. Michiels JJ, Berneman Z, Van Bockstaele D, van der Planken M, De Raeve H, Schroyens W. Clinical and laboratory features, pathobiology of platelet-mediated thrombosis and bleeding complications, and the molecular etiology of essential thrombocythemia and polycythemia vera: therapeutic implications. Semin Thromb Hemost 2006;32:174–207.
158. Elliott MA, Tefferi A. Thrombosis and haemorrhage in polycythaemia vera and essential thrombocythaemia. Br J Haematol 2005;128:275–290.
159. Landolfi R, Di gennaro L, Falanga A. Thrombosis in myeloproliferative disorders: pathogenetic facts and speculation. Leukemia 2008;22:2020–2028.
160. Di Nisio M, Barbui T, Di Gennaro L, et al. The haematocrit and platelet target in polycythemia vera. Br J Haematol 2007;136:249–259.
161. Cervantes F, Passamonti F, Barosi G. Life expectancy and prognostic factors in the classic BCR/ABL-negative myeloproliferative disorders. Leukemia 2008;22:905–914.
162. Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. Mayo Clin Proc 2006;81:159–166.

163. Finazzi G, Caruso V, Marchioli R, et al. Acute leukemia in polycythemia vera: an analysis of 1638 patients enrolled in a prospective observational study. Blood 2005;105:2664–2670.
164. Finazzi G, Barbui T. Risk-adapted therapy in essential thrombocythemia and polycythemia vera. Blood Rev 2005;19:243–252.
165. Berk PD, Goldberg JD, Donovan PB, Fruchtman SM, Berlin NI, Wasserman LR. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. Semin Hematol 1986;23:132–143.
166. Harrison CN. Platelets and thrombosis in myeloproliferative diseases. Hematology Am Soc Hematol Educ Program 2005: 409–415.
167. Carobbio A, Finazzi G, Guerini V, et al. Leukocytosis is a risk factor for thrombosis in essential thrombocythemia: interaction with treatment, standard risk factors, and Jak2 mutation status. Blood 2007;109: 2310–2313.
168. Landolfi R, Di Gennaro L, Barbui T, et al. Leukocytosis as a major thrombotic risk factor in patients with polycythemia vera. Blood 2007;109:2446–2452.
169. Carobbio A, Antonioli E, Guglielmelli P, et al. Leukocytosis and risk stratification assessment in essential thrombocythemia. J Clin Oncol 2008;26:2732–2736.
170. Antonioli E, Guglielmelli P, Pancrazzi A, et al. Clinical implications of the JAK2 V617F mutation in essential thrombocythemia. Leukemia 2005;19:1847–1849.
171. Wolanskyj AP, Lasho TL, Schwager SM, et al. JAK2 mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. Br J Haematol 2005;131:208–213.
172. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Clinical profile of homozygous JAK2V617F mutation in patients with polycythemia vera or essential thrombocythemia. Blood 2007;110:840–846.
173. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Prospective identification of high-risk polycythemia vera patients based on JAK2V617F allele burden. Leukemia 2007; 21:1952–1959.
174. Tefferi A, Huang J, Schwager S, et al. Validation and comparison of contemporary prognostic models in primary myelofibrosis: analysis based on 334 patients from a single institution. Cancer 2007;109: 2083–2088.
175. Cervantes F, Barosi G, Demory JL, et al. Myelofibrosis with myeloid metaplasia in young individuals: disease characteristics, prognostic factors and identification of risk groups. Br J Haematol 1998;102: 684–690.
176. Dupriez B, Morel P, Demory JL, et al. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. Blood 1996;88: 1013–1018.
177. Elliott MA, Verstovsek S, Dingli D, et al. Monocytosis is an adverse prognostic factor for survival in younger patients with primary myelofibrosis. Leuk Res 2007;31: 1503–1509.
178. Barosi G, Bergamaschi G, Marchetti M, et al. JAK2 V617F mutational status predicts progression to large splenomegaly and leukemic transformation in primary myelofibrosis. Blood 2007;110:4030–4036.
179. Tefferi A, Lasho TL, Huang J, et al. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. Leukemia 2008;22:756–761.
180. Barosi G, Mesa RA, Thiele J, et al. Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the international working group for myelofibrosis research and treatment. Leukemia 2008;22:437–438.
181. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 2005;90:1128–1132.
182. Manoharan A, Horsley R, Pitney WR. The reticulin content of bone marrow in acute leukaemia in adults. Br J Haematol 1979; 43:185–190.
183. Passamonti F, Rumi E, Caramella M, et al. A dynamic prognostic model to predict survival in post-polycythemia vera myelofibrosis. Blood 2008;111:3383–3387.
184. Tam CS, Nussenzveig RM, Popat U, et al. The natural history and treatment outcome of blast phase BCR-ABL negative myeloproliferative neoplasms. Blood 2008; 112:1628–1637.
185. Murphy S, Peterson P, Iland H, Laszlo J. Experience of the Polycythemia Vera Study Group with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment. Semin Hematol 1997;34:29–39.
186. Barbui T. The leukemia controversy in myeloproliferative disorders: is it a natural progression of disease, a secondary sequela of therapy, or a combination of both? Semin Hematol 2004;41:15–17.
187. Fruchtman SM, Mack K, Kaplan ME, Peterson P, Berk PD, Wasserman LR. From efficacy to safety: a Polycythemia Vera Study group report on hydroxyurea in patients with polycythemia vera. Semin Hematol 1997;34:17–23.
188. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. N Engl J Med 1995;332:1132–1136.
189. Barbui T, Finazzi G. When and how to treat essential thrombocythemia. N Engl J Med 2005;353:85–86.
190. Finazzi G, Barbui T. How I treat patients with polycythemia vera. Blood 2007;109: 5104–5111.
191. Tefferi A, Barbui T. bcr/abl-negative, classic myeloproliferative disorders: diagnosis and treatment. Mayo Clin Proc 2005;80: 1220–1232.
192. Harrison CN. Essential thrombocythaemia: challenges and evidence-based management. Br J Haematol 2005;130:153–165.
193. Barosi G, Bordessoule D, Briere J, et al. Response criteria for myelofibrosis with myeloid metaplasia: results of an initiative of the European Myelofibrosis Network (EUMNET). Blood 2005;106:2849–2853.
194. Tefferi A, Barosi G, Mesa RA, et al. International Working Group (IWG) consensus criteria for treatment response in myelofibrosis with myeloid metaplasia, for the IWG for Myelofibrosis Research and Treatment (IWG-MRT). Blood 2006; 108:1497–1503.
195. Streiff MB, Smith B, Spivak JL. The diagnosis and management of polycythemia vera in the era since the Polycythemia Vera Study Group: a survey of American Society of Hematology members' practice patterns. Blood 2002;99:1144–1149.
196. Brawley OW, Cornelius LJ, Edwards LR, et al. National Institutes of Health Consensus Development Conference statement: hydroxyurea treatment for sickle cell disease. Ann Intern Med 2008;148:932–938.
197. Barbui T, Barosi G, Grossi A, et al. Practice guidelines for the therapy of essential thrombocythemia. A statement from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica 2004;89:215–232.
198. Carobbio A, Finazzi G, Antonioli E, et al. Thrombocytosis and leukocytosis interaction in vascular complications of essential thrombocythemia. Blood 2008;112: 3135–3137.
199. Tefferi A, Gangat N, Wolanskyj AP. Management of extreme thrombocytosis in otherwise low-risk essential thrombocythemia; does number matter? Blood 2006; 108:2493–2494.
200. Budde U, Schaefer G, Mueller N, et al. Acquired von Willebrand's disease in the myeloproliferative syndrome. Blood 1984; 64:981–985.
201. Castaman G, Lattuada A, Ruggeri M, Tosetto A, Mannucci PM, Rodeghiero F. Platelet von Willebrand factor abnormalities in myeloproliferative syndromes. Am J Hematol 1995;49:289–293.
202. Kessler CM. Propensity for hemorrhage and thrombosis in chronic myeloproliferative disorders. Semin Hematol 2004;41:10–14.
203. Jabbour E, Kantarjian H, Cortes J, et al. PEG-IFN-alpha-2b therapy in BCR-ABL-negative myeloproliferative disorders: final result of a phase 2 study. Cancer 2007;110:2012–2018.
204. Samuelsson J, Hasselbalch H, Bruserud O, et al. A phase II trial of pegylated interferon alpha-2b therapy for polycythemia vera and essential thrombocythemia: feasibility, clinical and biologic effects, and impact on quality of life. Cancer 2006;106:2397–2405.
205. Silver RT. Long-term effects of the treatment of polycythemia vera with recombinant interferon-alpha. Cancer 2006;107: 451–458.
206. Kiladjian JJ, Cassinat B, Turlure P, et al. High molecular response rate of polycythemia vera patients treated with pegylated interferon alpha-2a. Blood 2006;108: 2037–2040.
207. Jones AV, Silver RT, Waghorn K, et al. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. Blood 2006;107:3339–3341.
208. Kiladjian JJ, Cassinat B, Chevret S, et al. Pegylated Interferon-alfa-2a induces complete hematological and molecular responses with low toxicity in Polycythemia Vera. Blood 2008;112:3065–3072.
209. Barbui T, Finazzi G. Myeloproliferative disease in pregnancy and other management issues. Hematology Am Soc Hematol Educ Program 2006:246–252.
210. Birgegard G. Anagrelide treatment in myeloproliferative disorders. Semin Thromb Hemost 2006;32:260–266.
211. Fruchtman SM, Petitt RM, Gilbert HS, Fiddler G, Lyne A. Anagrelide: analysis of long-term efficacy, safety and leukemogenic potential in myeloproliferative disorders. Leuk Res 2005;29:481–491.

212. Steurer M, Gastl G, Jedrzejczak WW, et al. Anagrelide for thrombocytosis in myeloproliferative disorders: a prospective study to assess efficacy and adverse event profile. Cancer 2004;101:2239–2246.

213. Barbui T, Finazzi G. Therapy for polycythemia vera and essential thrombocythemia is driven by the cardiovascular risk. Semin Thromb Hemost 2007;33:321–329.

214. Barosi G, Besses C, Birgegard G, et al. A unified definition of clinical resistance/intolerance to hydroxyurea in essential thrombocythemia: results of a consensus process by an international working group. Leukemia 2007;21:277–280.

215. Kroger N, Mesa RA. Choosing between stem cell therapy and drugs in myelofibrosis. Leukemia 2008;22:474–486.

216. Rondelli D, Barosi G, Bacigalupo A, et al. Allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning in intermediate- or high-risk patients with myelofibrosis with myeloid metaplasia. Blood 2005;105:4115–4119.

217. Byrne JL, Beshti H, Clark D, et al. Induction of remission after donor leucocyte infusion for the treatment of relapsed chronic idiopathic myelofibrosis following allogeneic transplantation: evidence for a 'graft vs. myelofibrosis' effect. Br J Haematol 2000;108:430–433.

218. Cervantes F, Rovira M, Urbano-Ispizua A, Rozman M, Carreras E, Montserrat E. Complete remission of idiopathic myelofibrosis following donor lymphocyte infusion after failure of allogeneic transplantation: demonstration of a graft-versus-myelofibrosis effect. Bone Marrow Transplant 2000;26:697–699.

219. Deeg HJ, Guardiola P. Allogeneic hemopoietic stem cell transplantation in patients with myelodysplastic syndrome or myelofibrosis. Int J Hematol 2002;76(suppl 2):29–34.

220. Guardiola P, Anderson JE, Bandini G, et al. Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Societe Francaise de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study. Blood 1999;93:2831–2838.

221. Barosi G, Bacigalupo A. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. Curr Opin Hematol 2006;13:74–78.

222. Ciurea SO, Sadegi B, Wilbur A, et al. Effects of extensive splenomegaly in patients with myelofibrosis undergoing a reduced intensity allogeneic stem cell transplantation. Br J Haematol 2008;141:80–83.

223. Li Z, Gooley T, Applebaum FR, Deeg HJ. Splenectomy and hemopoietic stem cell transplantation for myelofibrosis. Blood 2001;97:2180–2181.

224. Silverstein MN. Agnogenic myeloid metaplasia. Acton, Mass: Publishing Science Group; 1975.

225. Rodriguez JN, Martino ML, Dieguez JC, Prados D. rHuEpo for the treatment of anemia in myelofibrosis with myeloid metaplasia. Experience in 6 patients and meta-analytical approach. Haematologica 1998;83:616–621.

226. Cervantes F, Alvarez-Larran A, Hernandez-Boluda JC, et al. Darbepoetin-alpha for the anaemia of myelofibrosis with myeloid metaplasia. Br J Haematol 2006;134:184–186.

227. Cervantes F, Alvarez-Larran A, Hernandez-Boluda JC, Sureda A, Torrebadell M, Montserrat E. Erythropoietin treatment of the anaemia of myelofibrosis with myeloid metaplasia: results in 20 patients and review of the literature. Br J Haematol 2004;127:399–403.

228. Cervantes F, Hernandez-Boluda JC, Alvarez A, Nadal E, Montserrat E. Danazol treatment of idiopathic myelofibrosis with severe anemia. Haematologica 2000;85:595–599.

229. Cervantes F, Alvarez-Larran A, Domingo A, Arellano-Rodrigo E, Montserrat E. Efficacy and tolerability of danazol as a treatment for the anaemia of myelofibrosis with myeloid metaplasia: long-term results in 30 patients. Br J Haematol 2005;129:771–775.

230. Tefferi A, Elliot MA. Serious myeloproliferative reactions associated with the use of thalidomide in myelofibrosis with myeloid metaplasia. Blood 2000;96:4007.

231. Elliott MA, Mesa RA, Li CY, et al. Thalidomide treatment in myelofibrosis with myeloid metaplasia. Br J Haematol 2002;117:288–296.

232. Marchetti M, Barosi G, Balestri F, et al. Low-dose thalidomide ameliorates cytopenias and splenomegaly in myelofibrosis with myeloid metaplasia: a phase II trial. J Clin Oncol 2004;22:424–431.

233. Mesa RA, Elliott MA, Schroeder G, Tefferi A. Durable responses to thalidomide-based drug therapy for myelofibrosis with myeloid metaplasia. Mayo Clin Proc 2004;79:883–889.

234. Mesa RA, Steensma DP, Pardanani A, et al. A phase 2 trial of combination low-dose thalidomide and prednisone for the treatment of myelofibrosis with myeloid metaplasia. Blood 2003;101:2534–2541.

235. Tefferi A, Lasho TL, Mesa RA, Pardanani A, Ketterling RP, Hanson CA. Lenalidomide therapy in del(5)(q31)-associated myelofibrosis: cytogenetic and JAK2V617F molecular remissions. Leukemia 2007;21:1827–1828.

236. Lofvenberg E, Wahlin A. Management of polycythaemia vera, essential thrombocythaemia and myelofibrosis with hydroxyurea. Eur J Haematol 1988;41:375–381.

237. Naqvi T, Baumann MA. Myelofibrosis: response to busulfan after hydroxyurea failure. Int J Clin Pract 2002;56:312–313.

238. Petti MC, Latagliata R, Spadea T, et al. Melphalan treatment in patients with myelofibrosis with myeloid metaplasia. Br J Haematol 2002;116:576–581.

297. Tefferi A, Silverstein MN, Li CY. 2-Chlorodeoxyadenosine treatment after splenectomy in patients who have myelofibrosis with myeloid metaplasia. Brit J Haematol 1997;99:352–357.

240. Tefferi A, Mesa RA, Nagorney DM, Schroeder G, Silverstein MN. Splenectomy in myelofibrosis with myeloid metaplasia: a single-institution experience with 223 patients. Blood 2000;95:2226–2233.

241. Mesa RA, Nagorney DS, Schwager S, Allred J, Tefferi A. Palliative goals, patient selection, and perioperative platelet management: outcomes and lessons from 3 decades of splenectomy for myelofibrosis with myeloid metaplasia at the Mayo Clinic. Cancer 2006;107:361–370.

242. Houck WA, Mesa RA, Tefferi A. Antemortem presentation and management of non-hepatosplenic extramedullary hematopoiesis in myelofibrosis with myeloid metaplasia [abstract]. Blood 2000;96:747a.

243. Dingli D, Utz JP, Krowka MJ, Oberg AL, Tefferi A. Unexplained Pulmonary Hypertension in Chronic Myeloproliferative Disorders. Chest 2001;120:801–808.

244. Steensma DP, Hook CC, Stafford SL, Tefferi A. Low-dose, single-fraction, whole-lung radiotherapy for pulmonary hypertension associated with myelofibrosis with myeloid metaplasia. Br J Haematol 2002;118:813–816.

245. Bartlett RP, Greipp PR, Tefferi A, Cupps RE, Mullan BP, Trastek VF. Extramedullary hematopoiesis manifesting as a symptomatic pleural effusion. Mayo Clinic Proceedings 1995;70:1161–1164.

246. Pardanani A, Hood J, Lasho T, et al. TG101209, a small molecule JAK2-selective kinase inhibitor potently inhibits myeloproliferative disorder-associated JAK2V617F and MPLW515L/K mutations. Leukemia 2007;21:1658–1668.

247. Lasho TL, Finke C, Hood JD, et al. Primary cell experiments with TG101348, a JAK2-selective inhibitor, in the presence of myeloproliferative disorder-associated JAK2V617F, MPLW515L/K, and JAK2 Exon 12 Mutations [abstract]. Blood 2007;110:3541a.

286. Wernig G, Kharas MG, Okabe R, et al. Efficacy of TG101348, a Selective JAK2 inhibitor, in treatment of a murine model of JAK2V617F-induced polycythemia vera [abstract]. Blood 2007;110:556a.

249. Geron I, Abrahamsson A, Barroga C, et al. Selective inhibition of JAK2 driven erythroid differentiation of polycythemia vera progenitors [abstract]. Blood 2007;110:1526a.

250. Zaleskas VM, Chan WW, Evangelista P, et al. A selective and potent oral inhibitor of the JAK2 tyrosine kinase reverses polycythemia and leukocytosis induced by JAK2 V617F in a mouse model [abstract]. Blood 2007;110:557a.

251. Pardanani A. JAK2 inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. Leukemia 2008;22:23–30.

252. Guerini V, Barbui V, Spinelli O, et al. The histone deacetylase inhibitor ITF2357 selectively targets cells bearing mutated JAK2(V617F). Leukemia 2008;22:740–747.

253. Verstovsek S, Kantarjian H, Pardanani A, et al. INCB018424, an oral, selective JAK2 inhibitor, shows significant clinical activity in a phase i/ii study in patients with primary myelofibrosis (PMF) and post polycythemia vera/essential thrombocythemia myelofibrosis (Post-PV/ET MF) [abstract]. Blood 2007;110:558a.

254. Verstovsek S, Pardanani AD, Shah NP, et al. A phase I study of XL019, a selective JAK2 inhibitor, in patients with primary myelofibrosis and post-polycythemia vera/essential thrombocythemia myelofibrosis [abstract]. Blood 2007;110:553a.

255. Verstovsek S, Tefferi A, Kornblau S, et al. Phase II study of CEP701, an orally available JAK2 inhibitor, in patients with primary myelofibrosis and post polycythemia vera/essential thrombocythemia myelofibrosis [abstract]. Blood 2007;110:3543a.

256. Hexner EO, Serdikoff C, Jan M, et al. Lestaurtinib (CEP701)is a JAK2 inhibitor that suppresses JAK2/STAT5 signaling and the proliferation of primary erythroid cells from patients with myeloproliferative disorders. Blood 2008;111:5663–5671.