The myeloproliferative disorders comprise several clonal hematologic diseases that are thought to arise from a transformation in a hematopoietic stem cell. The main clinical features of these diseases are the overproduction of mature, functional blood cells and a long clinical course. Chronic myeloid leukemia (not discussed in detail here) is a myeloproliferative disorder that is defined by its causative molecular lesion, the BCR-ABL fusion gene, which most commonly results from the Philadelphia translocation (Ph). The three main Ph-negative myeloproliferative disorders—polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis—are the focus of this article. Less common conditions that are also considered myeloproliferative disorders under the classification used by the World Health Organization include systemic mastocytosis, chronic eosinophilic leukemia, chronic myelomonocytic leukemia, and chronic neutrophilic leukemia.

The cardinal features of the three main myeloproliferative disorders are an increased red-cell mass in polycythemia vera, a high platelet count in essential thrombocythemia, and bone marrow fibrosis in idiopathic myelofibrosis (Fig. 1). These three disorders share many characteristics, including marrow hypercellularity, a propensity to thrombosis and hemorrhage, and a risk of leukemic transformation in the long term. The annual incidences of both polycythemia vera and essential thrombocythemia are 1 to 3 cases per 100,000 population; myelofibrosis is less common.

**Pathogenetic Features**

**Clues to Molecular Mechanisms**

Polycythemia vera, a condition of “persistent and excessive hypercellularity accompanied by cyanosis,” was recognized by Vaquez in 1892, and idiopathic myelofibrosis was described at about the same time (Table 1). Essential thrombocythemia was recognized in the 1930s. Dameshek, in 1951, was the first to appreciate the considerable overlap in the clinical and laboratory features of these conditions and proposed that they, together with chronic myeloid leukemia and other rarer disorders, constitute a spectrum of related diseases. He coined the term “myeloproliferative disorders” to embrace these related conditions. In 1974, a critical experiment showed that erythroid progenitors from the bone marrow of patients with polycythemia vera were capable of growing in vitro in the absence of erythropoietin. At about the same time, studies based on X-chromosome inactivation were performed in women with polycythemia vera or essential thrombocythemia who carried a polymorphic variant of the X-linked G6PD gene. The results suggested that each of these diseases originated from a clone of hematopoietic stem cells.
Other clues to the molecular mechanisms responsible for the myeloproliferative disorders have been accumulating. These include the association of several chronic myeloid cancers with activated tyrosine kinases (Table 2)\cite{13-18,37} and the recognition of increased signaling through Janus kinases and signal transducers and activators of transcription (JAK–STAT) and phosphatidylinositol 3-kinase (PI3K) pathways in erythroid and myeloid cells.\cite{21,22,54} An additional hint came with the discovery of recurrent mitotic recombination events affecting chromosome 9p, resulting in the loss of heterozygosity but a normal DNA copy number.\cite{20}

**THE JAK2 V617F MUTATION**

In 2005, several groups reported a single, acquired point mutation in the Janus kinase 2 (JAK2) gene
in the majority of patients with Ph-negative myeloproliferative disorders.\textsuperscript{6-9,19} JAK2, a cytoplasmic tyrosine kinase, is critical for instigating intracellular signaling by the receptors for erythropoietin, thrombopoietin, interleukin-3, granulocyte colony-stimulating factor (G-CSF), and granulocyte–macrophage colony-stimulating factor (GM-CSF).\textsuperscript{55,56} Mice that are deficient in Jak2 die at embryonic day 12.5, with a complete absence of definitive erythropoiesis,\textsuperscript{57,58} a finding that underscores the vital role of JAK2 as a transducer of signals evoked by the binding of erythropoietin to its receptor. JAK2 binds to the erythropoietin receptor in the endoplasmic reticulum and is required for its cell-surface expression.\textsuperscript{59} When erythropoietin binds to its receptor, it provokes a conformational change in the receptor\textsuperscript{60-62} with consequent phosphorylation and activation of JAK2.\textsuperscript{63} The activated JAK2 then phosphorylates the receptor's cytoplasmic domain, thereby promoting the docking of downstream effector proteins and the initiation of intracellular signaling cascades\textsuperscript{55,56} (Fig. 2A).

The JAK2 mutation in the myeloproliferative disorders is not in the germ line but, rather, is acquired.\textsuperscript{6-9,19} Sensitive methods demonstrate the mutation in more than 95% of patients with polycythemia vera\textsuperscript{6-26} and in 50 to 60% of patients with essential thrombocythemia\textsuperscript{6-26,31,64} or idiopathic myelofibrosis.\textsuperscript{6,26,29-31} A substantial proportion of patients with polycythemia vera or idiopathic myelofibrosis are homozygous for the JAK2 mutation as a result of mitotic recombination affecting chromosome 9p.\textsuperscript{6-9} but this phenomenon is rarely detected in essential thrombocytemia.\textsuperscript{65} The mutation is also found in a small minority of patients with the hypereosinophilic syndrome, chronic myelomonocytic leukemia, chronic neutrophilic leukemia, myelodysplasia, or acute myeloid leukemia,\textsuperscript{31,34,66-68} but not in patients with lymphoid or other cancers or in those without hematologic disorders.\textsuperscript{6-9,33,34,67,69}

The presence of a mutant JAK2 in some patients with acute myeloid leukemia,\textsuperscript{33} especially older patients, raises the possibility that they may have had a preceding, undiagnosed myeloproliferative disorder. The JAK2 mutation is also found in more than 50% of patients with otherwise unexplained Budd–Chiari syndrome,\textsuperscript{70} which also suggests the presence of a masked myeloproliferative disorder in such cases.

The mutation in JAK2 substitutes a bulky phenylalanine for a conserved valine at position 617 of the JAK2 protein (V617F). This residue is located in the JH2, or pseudokinase, domain, which negatively regulates the kinase domain.\textsuperscript{71} Biochemical studies have shown that the JAK2 V617F mutation causes cytokine-independent activa-

<table>
<thead>
<tr>
<th>Year</th>
<th>Historical Milestone</th>
<th>Relationship Later Established with V617F Mutation in JAK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1892</td>
<td>First description of polycythemia vera\textsuperscript{4}</td>
<td>Mutation found in all three disorders\textsuperscript{5-9}</td>
</tr>
<tr>
<td>1951</td>
<td>Polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis linked as related conditions\textsuperscript{5}</td>
<td>Mutation associated with cytokine independence of primary erythroid progenitors and cell lines\textsuperscript{6-9}</td>
</tr>
<tr>
<td>1974</td>
<td>Identification of erythropoietin-independent erythroid colonies\textsuperscript{19}</td>
<td>Mutation found in multipotent progenitors and hematopoietic stem cells\textsuperscript{6-12}</td>
</tr>
<tr>
<td>1976</td>
<td>Stem-cell origin of polycythemia vera\textsuperscript{11}</td>
<td>Tyrosine kinase function of JAK2 constitutively activated by mutation\textsuperscript{7-9,19}</td>
</tr>
<tr>
<td>1983–2003</td>
<td>Dysregulated tyrosine kinases found in chronic myeloid leukemia,\textsuperscript{5,14} mastocytosis,\textsuperscript{15} chronic myelomonocytic leukemia,\textsuperscript{16,17} and chronic eosinophilic leukemia\textsuperscript{28}</td>
<td>Homozygosity of the mutation caused by mitotic recombination of chromosome 9p\textsuperscript{6-9}</td>
</tr>
<tr>
<td>2002</td>
<td>Description of mitotic recombination involving chromosome 9p as the most common cytogenetic lesion in polycythemia vera\textsuperscript{20}</td>
<td>STAT proteins constitutively activated by mutation\textsuperscript{7-9,19}</td>
</tr>
<tr>
<td>2001–2004</td>
<td>Erythropoietin-independent growth in polycythemia vera dependent on JAK–STAT signaling\textsuperscript{21,22}</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Description of the JAK2 V617F mutation\textsuperscript{6-9,19}</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{6} STAT denotes signal transducers and activators of transcription.
tion of JAK–STAT, PI3K, and AKT (also known as protein kinase B) pathways, and mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK),\textsuperscript{7,9,19} all of which are implicated in erythropoietin-receptor signaling.\textsuperscript{22,63,72,73}

The JAK2 mutation provides a unifying explanation for many features of the myeloproliferative disorders (Table 1), but other mutations are probably associated with JAK2 V617F-negative myeloproliferative disorders. One example is the presence of an activating mutation in the thrombopoietin receptor (MPL) gene in approximately 10% of patients with V617F-negative idiopathic myelofibrosis.\textsuperscript{35,36} This mutation is located in a motif that is important for keeping the receptor inactive.\textsuperscript{74}

### Pathophysiological Features

#### Genetic Marker

The insights revealed by the foregoing molecular investigations are reshaping our understanding of the pathophysiology, classification, diagnosis, and treatment of these conditions. The JAK2 mutation is the first genetic marker that is directly associated with the pathogenesis of the myeloproliferative disorders, and for this reason it is a powerful tool for analysis of the molecular and cellular basis of these disorders.

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent genetic lesions</td>
<td></td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>Chronic myeloid leukemia (100%)\textsuperscript{3}</td>
</tr>
<tr>
<td>JAK2 V617F</td>
<td>Polycythemia vera (95%),\textsuperscript{6,26} essential thrombocytopenia (50–60%),\textsuperscript{27,28} idiopathic myelofibrosis (50–60%),\textsuperscript{29,30} and other myeloid disorders (1–5%)\textsuperscript{31–34}</td>
</tr>
<tr>
<td>MPL W515K/L</td>
<td>Idiopathic myelofibrosis (5%) and essential thrombocytopenia (1%)\textsuperscript{35,36}</td>
</tr>
<tr>
<td>KIT mutations</td>
<td>Systemic mastocytosis\textsuperscript{15,37}</td>
</tr>
<tr>
<td>FIP1L1–PDGFRα</td>
<td>Chronic eosinophilic leukemia\textsuperscript{16,38}</td>
</tr>
<tr>
<td>PDGFRB fusion genes</td>
<td>Chronic myelomonocytic leukemia (rare)\textsuperscript{16}</td>
</tr>
<tr>
<td>FGFR fusion genes</td>
<td>Chronic myelomonocytic leukemia (rare)\textsuperscript{17}</td>
</tr>
<tr>
<td>Other cytogenetic lesions</td>
<td></td>
</tr>
<tr>
<td>Trisomy 9</td>
<td>Amplifies JAK2 gene and associated with JAK2 V617F mutation\textsuperscript{39}</td>
</tr>
<tr>
<td>Trisomy 8</td>
<td>Found in myeloproliferative disorders, myelodysplasia, and acute myeloid leukemias; target genes not identified</td>
</tr>
<tr>
<td>Trisomy 1q</td>
<td>Caused by duplication, trisomy, or unbalanced translocations\textsuperscript{40}</td>
</tr>
<tr>
<td>20q deletion</td>
<td>Found in myeloproliferative disorders and myelodysplasia\textsuperscript{41}; associated with JAK2 V617F mutation\textsuperscript{39} and precedes it in some cases\textsuperscript{42}; target genes not identified\textsuperscript{43}</td>
</tr>
<tr>
<td>5q and 7q deletions</td>
<td>Thought to reflect changes secondary to cytotoxic therapy\textsuperscript{40}; target genes not identified</td>
</tr>
<tr>
<td>13q deletion</td>
<td>Associated with idiopathic myelofibrosis; overlap of commonly deleted region and the region for chronic lymphocytic leukemia\textsuperscript{43}</td>
</tr>
<tr>
<td>Dysregulated genes and proteins</td>
<td></td>
</tr>
<tr>
<td>BCL-XL</td>
<td>Overexpressed in polycythemia vera\textsuperscript{43} as a result of constitutive JAK–STAT signaling; antiapoptotic effects in erythroid cells\textsuperscript{44,45}</td>
</tr>
<tr>
<td>NFE2</td>
<td>Up-regulated in JAK2-positive myeloproliferative diseases\textsuperscript{46,47}; may affect erythroid differentiation\textsuperscript{48,49}</td>
</tr>
<tr>
<td>PRV1</td>
<td>RNA levels increased in polycythemia vera\textsuperscript{50,51} but unlikely to play a direct role in disease pathogenesis since protein levels are not increased\textsuperscript{52}</td>
</tr>
<tr>
<td>MPL</td>
<td>Surface levels of protein decreased and aberrantly glycosylated in myeloproliferative disorders\textsuperscript{53,54}; role in disease pathogenesis unclear</td>
</tr>
</tbody>
</table>

\textsuperscript{6} FIP1L1 denotes FH interacting protein 1–like 1, PDGFRα platelet-derived growth-factor receptor alpha polypeptide, PDGFRβ platelet-derived growth-factor receptor beta polypeptide, FGFR fibroblast growth-factor receptor, BCL-XL B-cell leukemia/lymphoma 2–like protein X long-transcript variant, NFE2 nuclear factor erythroid–derived 2, PRV1 polycythemia rubra vera 1, and MPL thrombopoietin receptor.

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MOUSE MODELS
Expression of the JAK2 V617F mutation in murine hematopoietic cells by means of a retroviral vector recapitulates the features of polycythemia vera. The animals have erythrocytosis and leukocytosis, and there is evolution to postpolycythemic myelofibrosis. Thrombocytosis is not a reproducible feature in these mice, perhaps because high levels of mutant JAK2 generated by the retroviral vector inhibit the differentiation of megakaryocytes. Constitutive activation of signal transducers and activators of transcription (STAT5), erythropoietin hypersensitivity, and cytokine independence are all present, as in the human disease. These results provide direct evidence of a causal link between the mutation and the disease.

STEM-CELL BIOLOGY
The myeloproliferative disorders arise from a mutant multipotent stem cell. Analyses of patterns of X-chromosome inactivation in female patients show a skewed pattern in myeloid cells but a balanced pattern in T cells and other cell types, suggesting that some myeloid cells in these women are clonally derived. However, skewed patterns of X-chromosome inactivation in granulocytes have been found in older women without a myeloproliferative disorder and are thought to reflect polymorphic X-linked genes that influence hematopoietic stem-cell behavior. Age-related skewing limits the use of the analysis of X-chromosome inactivation for diagnostic purposes, but such studies remain useful as research tools. For example, studies of X-chromosome inactivation indicate that in most patients with V617F-negative essential thrombocythemia or idiopathic myelofibrosis, there is a dominant clone of blood cells, implying that these disorders are clonal hematologic diseases.

The presence of the JAK2 mutation in colonies of hematopoietic progenitor cells (grown in vitro) and in hematopoietic stem cells isolated by fluorescence-activated cell sorting provides direct evidence that polycythemia vera and related myeloproliferative disorders arise in a multipotent progenitor or stem cell. In contrast to the normal-size myeloid stem-cell compartment in chronic myeloid leukemia, the stem-cell compartment in polycythemia vera is both expanded and characterized by preferential erythroid differentiation. Taken together, the data suggest that the JAK2 mutation affects the behavior of hematopoietic stem cells but probably also acts at later stages of differentiation, particularly in the erythroid, granulocytic, and megakaryocytic lineages (Fig. 2B).

COOPERATING MUTATIONS
The JAK2 mutation explains many of the cardinal features of the myeloproliferative disorders, as does the BCR-ABL fusion gene in chronic myeloid leukemia. It seems likely that, as in chronic myeloid leukemia, leukemic transformation of the myeloproliferative disorders reflects the acquisition of additional mutations.

Four lines of evidence suggest that cooperating mutations occur at an early stage in V617F-positive myeloproliferative disorders and can even predate the JAK2 mutation. First, deletions of 20q and other cytogenetic abnormalities occur in 5 to
10% of patients with a myeloproliferative disorder. In one patient with polycythemia vera and in one with essential thrombocythemia, granulocytes with the 20q deletion outnumbered V617F-positive granulocytes, suggesting that the 20q deletion occurred before the JAK2 mutation. Patients with molecularly defined 20q deletions are almost exclusively V617F-positive, which is consistent with the presence of a cooperating gene on 20q. Second, some women with polycythemia vera or essential thrombocythemia have more clonally derived granulocytes (estimated by patterns of X-chromosome inactivation) than JAK2-positive granulocytes. Although this finding has been interpreted as evidence of a clonal proliferation that preceded the JAK2 mutation, such a conclusion remains controversial.

Third, in some patients with V617F-positive polycythemia vera or essential thrombocythemia that undergoes leukemic transformation, the leukemic cells lack the JAK2 mutation. This finding is consistent with the origin of the leukemia in a mutant clone that preceded the JAK2 mutation, although other interpretations are possible. Fourth, in familial clusters of the myeloproliferative disorders, the JAK2 mutation is acquired, which suggests that the inherited predisposition is unrelated to the JAK2 mutation.

**Homozygosity for V617F**

Homozygosity for the V617F mutation plays a key role in the myeloproliferative disorders. Homozygosity results from mitotic recombination (Fig. 2C), with the breakpoints spread along chromosome 9p between the JAK2 locus and the centromere, implying that there is no single fragile site that is prone to recombination. In polycythemia vera, the prevalence of blood cells that are homozygous for the JAK2 mutation increases with time, presumably owing to a proliferative or survival advantage of mutant progenitor cells. Homozygosity for V617F is associated with more marked changes in the expression of downstream target genes than is heterozygosity for V617F. This reflects increased signaling mediated by a double dose of V617F, possibly combined with a loss of inhibition by the wild-type allele.

Homozygosity in granulocytes can be detected in about 30% of patients with polycythemia vera. However, when colonies of hematopoietic progenitor cells derived from such patients were studied, approximately 90% of them were homozygous for the V617F mutation. By contrast, homozygous progenitor colonies were not found in essential thrombocythemia. This difference suggests that V617F homozygosity promotes the development of polycythemia vera. Since there is substantial variation in the rate of mitotic recombination among persons without a myeloproliferative disorder, genetic and environmental factors that determine susceptibility to mitotic recombination may influence whether essential thrombocythemia or polycythemia vera develops.

**Signaling Pathways**

The mutant JAK2 protein activates multiple downstream signaling pathways with effects on gene transcription, apoptosis, the cell cycle, and differentiation (Table 2). Effects on apoptosis include overexpression of the cell-survival protein BCL-X in erythroid precursor cells in polycythemia vera, probably as a result of enhanced JAK–STAT signaling. There is also a reduction in apoptosis induced by death receptors in JAK2 V617F-positive erythroid progenitors, an effect mediated through PI3K–AKT and MAPK–ERK pathways. With respect to the cell cycle, mutant JAK2 promotes G1/S phase transition in hematopoietic cell lines, accompanied by up-regulation of cyclin D2 and down-regulation of the inhibitor p27kip.

Effects on erythroid differentiation may be mediated by nuclear factor erythroid-derived 2 (NF-E2), which is up-regulated in polycythemia vera and plays an important role in erythroid differentiation. Hematopoietic cells expressing JAK2 V617F become hypersensitive to insulin-like growth factor 1 (IGF-1), suggesting that IGF-1 receptor signaling influences cellular proliferation and differentiation in these diseases.

Wild-type JAK2 is required for intracellular processing and cell-surface display of the erythropoietin receptor and the thrombopoietin receptor. Mutant JAK2 influences the display and stability of the erythropoietin receptor on the cell surface but reduces levels of cell-surface thrombopoietin receptor (MPL) in cultured cells (personal communication). The latter may explain the decreased levels and altered glycosylation of MPL in the myeloproliferative disorders.
a type-1 cytokine receptor — such as the erythropoietin receptor (EPOR), MPL, or G-CSF receptor — to act as a scaffold and docking site for downstream effector proteins. Such a mechanism may explain the involvement of the erythroid, megakaryocyte, and granulocyte lineages in the myeloproliferative disorders (Fig. 2B), but detailed structural and biochemical analyses are needed to explain why residue 617 is so critical for JAK2 activation.

**DISEASE EVOLUTION**

The evolution of the myeloproliferative disorders into other forms is poorly understood. An increase in reticulin fibrosis may occur with time in patients with polycythemia vera or essential thrombocythemia, and in some of them a syndrome indistinguishable from idiopathic myelofibrosis develops. The stromal cells and fibroblasts responsible for the increased fibrosis, angiogenesis, and formation of new bone do not derive from the myeloproliferative clone. They therefore represent a polyclonal reaction to cytokines — including transforming growth factor β (TGF-β), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) — produced by megakaryocytes, monocytes, or both from the myeloproliferative clone. Mouse models of myelofibrosis suggest that excessive MPL signaling, activation of nuclear factor κB (NF-κB), and low levels of the globin transcription factor 1 (GATA-1) also contribute to cytokine secretion and the development of reticulin fibrosis. In V617F-positive human disease, excessive MPL signaling may be particularly relevant, since the JAK2 protein is a key component of signal transduction from the MPL receptor.

Acute myeloid leukemia develops in a small minority of patients with a myeloproliferative disorder, but transformation to acute lymphoblastic leukemia is extremely rare. Leukemic transformation can occur without exposure to cytoxic agents and probably reflects acquisition of additional genetic lesions. The development of acute myeloid leukemia in the absence of the JAK2 mutation in patients with a V617F-positive myeloproliferative disorder demonstrates that mutant JAK2 is not necessarily involved in leukemic transformation.

**MOLECULAR CLASSIFICATION**

Prospective and retrospective studies of essential thrombocythemia have shown that the V617F mutation divides the disease into biologically distinct subtypes. V617F-positive thrombocythemia resembles polycythemia vera. In contrast to V617F-negative essential thrombocythemia, V617F-positive thrombocythemia typically shows higher levels of hemoglobin and white cells, a more cellular bone marrow, an increased risk of venous thrombosis and transformation to polycythemia vera, and greater sensitivity to hydroxyurea. These features suggest that V617F-positive thrombocythemia is a forme fruste of polycythemia vera, with the degree of erythrocytosis influenced by factors such as low iron stores, low erythropoietin levels, sex, and homozygosity for V617F. Fifty patients with V617F-negative essential thrombocythemia remained V617F-negative for more than 6 years, indicating that this disorder is distinct from, and not an early “pre-JAK2” phase of, V617F-positive thrombocythemia.

V617F-positive patients with idiopathic myelofibrosis had a reduced transfusion requirement and greater white-cell counts than did patients without the mutation, analogous to the higher hemoglobin levels and white-cell counts in patients with V617F-positive essential thrombocythemia. In this study, but not in a second, the V617F mutation was independently associated with poor survival.

These results lay the foundation for a molecular classification of the myeloproliferative disorders (Fig. 3). This classification is likely to evolve as additional genetic lesions are identified within the JAK2 V617F-negative category. The similarities between V617F-positive essential thrombocythemia and polycythemia suggest considerable overlap between these conditions. They may form a phenotypic continuum in which the degree of initial erythrocytosis or thrombocytopoiesis depends on physiological or genetic modifiers.
The disorder is analogous to the accelerated phase of chronic myeloid leukemia and is probably spurred by the acquisition of additional genetic changes. In the myeloproliferative disorders, the accelerated phase would encompass not only the transformation of polycythemia vera or essential thrombocythemia into myelofibrosis, but also a rising white-cell count, increased blasts in the blood, and neutropenia or thrombocytopenia. It is important to distinguish transformation to myelofibrosis from histologic detection of increased levels of reticulin. The former is a clinical syndrome indicating a biologic change within the myeloproliferative clone, whereas the latter may accompany chronic-phase polycythemia vera or essential thrombocythemia, with the degree of fibrosis reflecting an interplay between the duration of the disease and physiological or genetic modifiers.

The disorder that is currently termed idiopathic myelofibrosis is clinically indistinguishable from the transformation of polycythemia vera or essential thrombocythemia to myelofibrosis, and the diagnostic criteria for the two conditions are similar. At least some patients who receive the diagnosis of idiopathic myelofibrosis...
probably are in the accelerated phase of previously unrecognized polycythemia vera or essential thrombocythemia.

**DIAGNOSIS**

Until recently, robust tools for the diagnosis of the myeloproliferative disorders have been lacking. Criteria for the diagnosis of polycythemia vera are mostly modifications of 20-year-old standards from the Polycythemia Vera Study Group. The available tests are expensive, not universally available, and lacking in sensitivity and specificity. They include a determination of red-cell mass to distinguish true erythrocytosis from relative polycythemia, identification of erythropoietin-independent erythroid colonies in vitro, cytogenetic analysis of bone marrow cells, testing of erythropoietin levels, ultrasonography of the spleen, and testing for the overexpression of polycythemia rubra vera (PRV). There are no universally accepted diagnostic tests for essential thrombocythemia.

Testing for the JAK2 V617F mutation is now widely available and promises to simplify the diagnostic workup. Allele-specific polymerase-chain-reaction (PCR) assay, restriction-enzyme digestion, and real-time PCR are all sufficiently sensitive to detect the presence of a heterozygous mutation in as few as 5 to 10% of cells. These assays have low rates of false positive results, making them useful diagnostic tools.

Proposed diagnostic criteria for V617F-positive and V617F-negative myeloproliferative disorders are outlined in Tables 3 and 4, respectively. The JAK2 mutation is not associated with causes of an absolute erythrocytosis other than polycythemia vera. Therefore, in the absence of a coexisting secondary erythrocytosis, the presence of the JAK2 mutation in a patient with an increased red-cell mass is sufficient for the diagnosis of polycythemia vera. In this situation, the role of analysis of the red-cell mass is mainly to distinguish polycythemia vera from essential thrombocythemia. However, this distinction is becoming arbitrary, given the clinical and biologic similarities between the two conditions.

Therefore, in patients with the JAK2 mutation, analysis of the red-cell mass is likely to become obsolete. Erythropoietin levels do not distinguish between polycythemia vera and V617F-positive essential thrombocythemia and therefore have minimal use in patients with the JAK2 mutation. Cytogenetic studies in patients with the JAK2 mutation generally do not add useful diagnostic or prognostic information.

V617F-negative polycythemia vera represents less than 5% of all cases of polycythemia vera. In these rare cases, apparent erythrocytosis should be excluded by analysis of the red-cell mass and secondary erythrocytosis by measurement of erythropoietin levels and oxygen saturation. Abnormal results on cytogenetic analysis or radiologic evidence of splenomegaly would support the diagnosis of a myeloproliferative disorder.

The presence of a JAK2 mutation in a patient with thrombocythiosis has a high positive predictive value for a myeloproliferative disorder; patients with reactive or secondary thrombocythiosis and persons without a hematologic disorder are V617F-negative (Table 3). Other causes of throm-
bocytosis must be excluded in patients without the mutation (Table 4). Cytogenetic analysis of bone marrow is sometimes helpful in JAK2-negative thrombocytocmia if it shows a clonal abnormality. Essential thrombocytocmia has been divided into histologically distinct subgroups (labeled “true” essential thrombocytocmia, prefibrotic myelo-fibrosis, and early overt myelofibrosis) that are thought to have differing prognoses, but it is unclear whether this histologic classification is robust enough to be applied outside specialized centers.

Diagnostic criteria for idiopathic myelofibrosis are presented in Tables 3 and 4, modified from the Italian Consensus Conference. The criteria proposed here define a clinical syndrome in which reticulin fibrosis of the marrow is accompanied by specific clinical or laboratory features and can be used for both idiopathic myelofibrosis and transformation from preceding essential thrombocytocmia or polycythocmia vera.

**Table 4. Proposed Diagnostic Criteria for Myeloproliferative Diseases without JAK2 Mutation.**

<table>
<thead>
<tr>
<th>JAK2-negative polycythocmia vera (diagnosis requires the presence of A1, A2, and A3, plus either another A criterion or two B criteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1. Increased red-cell mass (&gt;25% above predicted value) or a hematocrit ≥60% in men or &gt;56% in women</td>
</tr>
<tr>
<td>A2. Absence of mutation in JAK2</td>
</tr>
<tr>
<td>A3. No causes of secondary erythrocytosis (normal arterial oxygen saturation and no elevation of serum erythropoietin)</td>
</tr>
<tr>
<td>A4. Palpable splenomegaly</td>
</tr>
<tr>
<td>A5. Presence of acquired genetic abnormality (excluding BCR-ABL) in hematopoietic cells</td>
</tr>
<tr>
<td>B1. Thrombocytosis (platelets &gt;450×10⁹/liter)</td>
</tr>
<tr>
<td>B2. Neutrophilia (neutrophils &gt;10×10⁹/liter; &gt;12.5×10⁹/liter in smokers)</td>
</tr>
<tr>
<td>B3. Splenomegaly on radiography</td>
</tr>
<tr>
<td>B4. Endogenous erythroid colonies or low serum erythropoietin</td>
</tr>
<tr>
<td><strong>JAK2-negative essential thrombocytocmia</strong> (diagnosis requires the presence of all five criteria)</td>
</tr>
<tr>
<td>A1. Platelet count &gt;600×10⁹/liter on two occasions at least 1 mo apart</td>
</tr>
<tr>
<td>A2. Absence of mutation in JAK2</td>
</tr>
<tr>
<td>A3. No reactive cause for thrombocytosis</td>
</tr>
<tr>
<td>A4. Normal ferritin (&gt;20 μg/liter)</td>
</tr>
<tr>
<td>A5. No other myeloid disorder, especially chronic myeloid leukemia, myelofibrosis, polycythocmia vera, or myelodysplasia</td>
</tr>
<tr>
<td><strong>JAK2-negative idiopathic myelofibrosis</strong> (diagnosis requires the presence of A1, A2, A3, and any two B criteria)</td>
</tr>
<tr>
<td>A1. Reticulin grade 3 or higher (on a 0–4 scale)</td>
</tr>
<tr>
<td>A2. Absence of mutation in JAK2</td>
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<tr>
<td>A3. Absence of BCR-ABL fusion gene</td>
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<tr>
<td>B1. Palpable splenomegaly</td>
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<tr>
<td>B2. Otherwise unexplained anemia (hemoglobin &lt;11.5 g/liter for men or &lt;10 g/liter for women)</td>
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<tr>
<td>B3. Teardrop red cells on peripheral blood film</td>
</tr>
<tr>
<td>B4. Leukerythroblastic blood film (presence of at least 2 nucleated red cells or immature myeloid cells in peripheral blood film)</td>
</tr>
<tr>
<td>B5. Systemic symptoms (drenching night sweats, weight loss &gt;10% over 6 mo, or diffuse bone pain)</td>
</tr>
<tr>
<td>B6. Histologic evidence of extramedullary hematopoiesis</td>
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</tbody>
</table>

*The platelet threshold is preferred in patients without the JAK2 mutation, given the difficulty in ruling out reactive thrombocytosis and the fact that 2.5% of persons without a myeloproliferative disorder have a platelet count above the normal range.*

**TREATMENT**

The JAK2 mutation is reshaping how we treat the myeloproliferative disorders. Moving to a new classification could simplify therapy. In polycythocmia vera, the hematocrit is the primary target of therapy, but the treatment of patients with thrombocytosis is controversial. By contrast, in essential thrombocytocmia, cytoreduction to normalize the platelet count reduces thrombosis in high-risk patients, but the hematocrit is not a therapeutic target. Given the many similarities between JAK2-positive polycythocmia and thrombocytocmia, it seems logical to develop target levels for both the platelet count and the hematocrit in both conditions. The development of a unified treatment strategy is consistent with growing evidence that the increased risk of thrombosis is not exclusively caused by erythrocytosis or thrombocytosis but by interactions among white cells, red cells, platelets, and the endothelium. Abnormalities in the activation of leukocytes and platelets occur in both diseases.

The JAK2 mutation also affects response to treatment. Among patients with essential thrombocytocmia, those with the V617F mutation are more sensitive to hydroxyurea (but not to anagrelide) than are patients without the mutation. The response to therapy can now be directly monitored through quantification of JAK2-positive cells in peripheral blood.

An understanding of the molecular pathogenesis of the myeloproliferative disorders lays the foundation for the development of novel targeted therapies. Since JAK2 inhibitors reduce the growth of JAK2 V617F-positive cell lines and primary cells in vitro, there is considerable interest in de-
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Developing compounds for clinical use. Such agents hold particular promise for the treatment of patients whose disease is in the accelerated phase, including myelofibrosis, given the lack of satisfactory treatments currently available. These agents may also prove to be useful for reducing the risk of vascular events or long-term disease evolution in chronic-phase disease. With an eye to the success of imatinib in the treatment of chronic myeloid leukemia, we hope the next decade will see the development of well-tolerated, therapeutic JAK2 inhibitors.

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