Myeloproliferative neoplasms represent a family of clonal hematopoietic stem cell disorders that exhibit a wide variety of clinical, biologic, and phenotypic heterogeneity. MPNs serve as the model of acquired somatic genetic lesions as the causal basis of cancer (so-called drivers), from the first genetic lesion ascribed to cancer, the Philadelphia (Ph) chromosome in chronic myelogenous leukemia (CML), to the more recent discoveries of lesions in JAK2, MPL, and CALR in what were previously called Ph-chromosome–negative MPN. MPNs cause a substantial amount of morbidity and mortality during a patient’s lifetime, including increased cardiovascular, thrombotic, and hemorrhagic events, and increased lifetime risk for transformation to acute myeloid leukemia (AML), bone marrow failure, or MF. In the 10 years since the JAK2V617F discovery, the MPN field has experienced a profound increase in both clinical and basic research with regard to further understanding of the pathobiologic basis for the disease, especially illustrated by the more recent discoveries of diagnostic and prognostic significance of JAK2, MPL, CALR, ASXL1, and other molecular markers. Importantly, genetic discovery in CML and MPN have been translated to effective, targeted therapy, capable of altering the natural history of the MPN. This review will focus on the most recent advances in risk stratification of the patient with MPN, and how risk stratification can be translated to disease- and patient-specific therapy.

ACQUIRED SOMATIC MUTATION IN THE MPN: IDENTIFICATION OF CAUSAL LESIONS

Chronic myeloproliferative disorders (MPDs) were recognized as clinical entities in the 19th century, but the 20th century brought precise clinical classification and their genetic basis into being. The 21st century has, and continues to, unravel their causal genetic basis. The primary nature of bone marrow’s overproduction was compared with that of leukemia, and both CML and PV were considered as neoplasms of the hematopoietic tissue. William Dameshek theorized that these entities might be driven by a shared stimulus intrinsic to the marrow itself, and coined the term MPDs.7 The identification of the Ph chromosome as specific to CML and not to the rest of the MPD class was not only an advance in disease classification within MPNs, but, ultimately, confirmed the causal association of an acquired genetic lesion in the generation of human cancer.8 Given the similarities of Ph-chromosome–negative MPN to Ph-positive–MPN (stem cell basis, overproduction of mature myeloid elements, tendency to evolve to MF or acute leukemia), lesions in tyrosine kinase pathways critical to erythropoietin, thrombopoietin, and granulocyte-colony stimulating factor signal transduction were investigated. Advances in DNA sequencing technology led several investigators to resequence the JAK2 gene in patients with PV because of its specific activity in primary PV cells, its identity as a ty-
rosine kinase, or its location in the chromosome 9p loss of heterozygosity region highly prevalent in MPNs.9-12 JAK2 mutations are identified in nearly 100% of patients with PV, and are also highly prevalent in patients with thrombocytosis (ET) and primary MF (PMF; Table 1). Like BCR-ABL1, evidence that driver lesions are causative in MPNs are as follows: recapitulation of MPN disease in transgenic murine models,13-15 gene-dosage effect of JAK2V617F on MPN phenotype,16 and hematologic remissions corresponding to molecular remissions.4,5 The BCR-ABL and JAK2 discoveries also have hastened the discovery of other causative MPN lesions by allowing for enrichment of lesion-negative MPNs in global resequencing studies.14,17-19 Table 1 outlines the high-frequency causal driver lesions identified to date and their relative frequency in MPNs. Given the relative prevalence of the various MPNs and the frequency of driver lesions within MPNs, JAK2V617F is the most common lesion in this group. The term Ph-chromosome–negative MPN was applied because the Ph discovery was the first. This term is no longer relevant as identified lesions in Table 1 now have come to define their clinical entities.

OVERVIEW OF RISK ASSESSMENT IN MPNs
In contrast to CML, a disease with a fairly uniform sequential natural history driven by BCR-ABL1, marked variability exists in the natural history of JAK2-, CALR-, MPL-, and KIT-mutation–positive disease. For instance, JAK2V617F in many individuals is latent and may be asymptomatic or aphenotypic for undisclosed periods, as evidenced by its detection in large unselected population surveys.20 Moreover, cells that have acquired JAK2V617F often acquire an additional copy of JAK2V617F via mitotic recombination events, such that JAK2V617F mutation dosage is a variable to be accounted for both at diagnosis and during disease monitoring. In these diseases, particularly in JAK2V617F–positive MPN, factors such as age at diagnosis, sex, treatment exposure, host genetic background, gene dosage, and other acquired genetic lesions, to a large part, contribute to risk for disease acquisition,21,22 disease evolution or progression, and thrombosis.23,24 Thus, risk stratification in MPNs must take into account disease class, specific genetic lesions, lesion burden, and host factors and requires large observational prospective cohorts to establish risk. Estimating risk throughout decades of disease is challenging and often confounded by the individual, and by the lack of knowledge of somatic mutations and their roles. In diseases where survival is measured in decades, risk assessment includes not only long-term risks of disease evolution or transformation, but also ongoing symptom burden risk assessment.

#### RISK STRATIFICATION IN MPNs

##### Essential Thrombocytosis

ET is an MPN that features a risk for morbidity and mortality secondary to either thrombotic or hemorrhagic events, as well as transformation to PV, MF, and AML, and represents a distinct entity from PV.25 Historically, underlying molecular abnormalities of ET remained elusive until discovery of the JAK2V617 mutation (present in 50% to 60% of cases), MPL mutations (4% of cases), and, most recently, CALR mutations (15% to 24% of cases; Table 1).26,27 In terms of thrombosis risk stratification in the clinic, a dual risk factor system has been used based on older age of the patient and a personal history of prior thrombotic (or hemorrhagic) events.28 Several groups have subsequently put forward additional clinical and laboratory features to be considered. In 2012, the International Working Group for Myeloproliferative Neoplasms Research and Treatment reported on the International Prognostic Score for Essential Thrombocythosis (IPSET).29 This study analyzed 867 patients with World Health Organization (WHO)-defined ET and found that three factors informed prognosis by multivariate analysis: (1) age 60 or older, (2) white blood cells (WBC) of 11K/uL or greater, and (3) personal history of a thrombotic event. Ultimately, patients were put into one of three risk groups: low, intermediate, and high risk. The IPSET model was able to predict significant differences in survival (median overall survival: low risk, not reached; intermediate risk, 24.5 years; and high risk, 13.8 years). Additionally, the model had the ability to predict thrombotic event rate occurrence. It was unable to account for transformation to MF.

Another recent ET scoring system from a retrospective study focused on the risk for thrombosis in patients with ET, termed the IPSET-thrombosis model.30 This important
TABLE 2. Thrombotic Risk Factors Associated with the Myeloproliferative Neoplasm

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPN class</td>
<td>PV &gt; MF &gt; ET</td>
</tr>
<tr>
<td>Prior thrombotic event</td>
<td></td>
</tr>
<tr>
<td>Presence of cardiovascular</td>
<td></td>
</tr>
<tr>
<td>Age &gt; 60</td>
<td></td>
</tr>
<tr>
<td>Hematocrit &gt; 45%</td>
<td>Validated in PV</td>
</tr>
<tr>
<td>JAK2V617F</td>
<td>Strongly associated with BCS</td>
</tr>
</tbody>
</table>

Abbreviations: MPN, myeloproliferative neoplasm; PV, polycythemia vera; ET, essential thrombocytosis; BCS, Budd-Chiari syndrome.

TABLE 3. Risk Factors for Disease Evolution in Myeloproliferative Neoplasm

<table>
<thead>
<tr>
<th>Evolution</th>
<th>Clinical Risk Factors</th>
<th>Genetic Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET to PV</td>
<td>Disease duration</td>
<td>JAK2V617F, JAK2V617F allele %</td>
</tr>
<tr>
<td>ET to MF</td>
<td>Disease duration, male sex, age</td>
<td>JAKV61F allele %, ASXL1, 20q deletion, 13q deletion, trisomy 8</td>
</tr>
<tr>
<td>PV to PPVMF</td>
<td>Disease duration</td>
<td>Trisomy 9, ASXL1, 20q deletion, 13q deletion, trisomy 8</td>
</tr>
<tr>
<td>MPN to AML</td>
<td>Age, disease duration, karyotypic complexity, myelofibrosis phase, P32, alkylator exposure</td>
<td>JAK2V617F allele %, complex karyotypic lesions, TP53, RUNX1 lesions</td>
</tr>
</tbody>
</table>

Abbreviations: ET, essential thrombocytosis; PV, polycythemia vera; MF, myelofibrosis; PPVMF, postpolycythemia vera myelofibrosis; MPN, myeloproliferative neoplasm; AML, acute myeloid leukemia.

model was validated with internal and external datasets, again demonstrating improved prognostication for risk for thrombosis among patients with ET. Among 891 patients with WHO-classified ET, multivariate analysis and risk scoring found four factors to be significant in terms of predicting thrombotic risk: (1) age older than 60, (2) prior thrombotic history, (3) cardiovascular risk factors, and (4) JAK2V617F mutation positivity. The model demonstrated three distinct at-risk thrombotic groups: low risk, 1.03% of patients per year; intermediate risk, 2.35% of patients per year; and high risk, 3.56% of patients per year. Notably in this model, the presence of WBC count is removed as a significant factor, and, instead, both cardiovascular risk factors and the JAK2V617F mutation are added and demonstrate significance. As this model is based on retrospective data, the prospective validation of this model will be warranted and the presence of leukocytosis and presence of JAK2V617F mutation and its corresponding allele burden and overall thrombotic risk will need to be further explored, as prior reports have demonstrated that a higher JAK2 allele burden may not necessarily correlate with a higher thrombotic risk in all patients with ET.31

In a recent study of 1,150 patients with ET from four Italian centers, Finazzi et al retrospectively sought to analyze if the presence of the newly defined CALR mutation would affect prediction of thrombosis by the IPSET-thrombosis model.32 Among the 1,150 patients, 164 patients (14%) were found to be CALR mutated. There was a higher incidence of CALR mutation among the low- and intermediate-risk groups as compared with the high-risk IPSET group, and there was a trend toward lower rate of thrombosis noted in the patients with CALR-mutated disease as compared with patients with JAK2V617-mutated disease (1.30% vs. 1.95% of patients per year), but this was not significant. This finding, as noted by the authors, was mitigated by the fact that CALR mutation was also more commonly found among patients with ET with fewer prior thrombotic events and younger patients (Table 2).

ET, regardless of genetic driver, carries significant risks during the lifetime of a patient for transformation to PV and MF. Transformation to PV is a function of time and is closely associated with JAK2V617F-positive status, in addition to increasing JAK2V617F allele percentage. Post-ET MF, in contrast, occurs in all ET settings, and is associated with not only disease duration, but also acquisition of additional genetic lesions and male sex (Table 3).33

Polycythemia Vera

Historically, risk stratification in PV incorporated age of the patient (older than 65) and prior thrombotic history.34 More recently, Tefferi et al reviewed outcomes among a large group of patients (1,545 patients) with WHO-specified PV.24 In this multivariate analysis, the authors found that leukocytosis, advanced age, abnormal cytogenetics, and history of a venous thrombotic event portend a more adverse outcome in terms of overall survival. This model system also further analyzed factors important for predicting transformation to AML, and found that three out of the four factors for survival were still significant for this outcome (all but venous thrombotic event; Tables 2 and 3).

About 20% of patients with PV may experience a thrombotic event during the course of their disease. Thrombotic events occur on the arterial and venous circulation, microvascular, or thromboembolic, such as transient ischemic attacks, myocardial infarction, deep venous thrombosis, or pulmonary embolism. Venous thrombosis in unusual circulations may occur in the patient with PV, particularly in the portal and hepatic (splanchnic), splenic vein, and cerebral venous sinuses. An important element in risk stratification of the patient with PV is a comprehensive assessment of a patient’s underlying risk factors for thromboembolism, including an understanding of the patient’s cardiovascular risk factors, including smoking use and history.35 The importance of hematocrit control, in addition to controlling traditional cardiovascular risks, was established by the CYTO-PV Collaborative Group, whereby an aggressively pursued threshold of hematocrit 45 or greater resulted in a three- to four-times risk reduction in terms of cardiovascular morbidity and mortality, as compared with the group with less strict hematocrit regulation.36

Thrombotic risk in MPNs has also led to risk stratification in patients with occurrence of idiopathic Budd-Chiari syn-
drome (BCS) and portal vein thrombosis (PVT). In a series by Kiladjian et al, 241 patients with splanchic vein thrombosis (SVT) were examined. JAK2V617F mutation was found in 45% of the BCS cases, and 34% of the PVT cases; whereas, JAK2 exon 12 and MPL mutations were not identified in any. A meta-analysis showed that because of the close association of JAK2V617F-positive MPN in patients with new SVTs, routine screening with JAK2V617F mutation was warranted in all patients presenting with idiopathic SVTs, even in patients where MPN phenotype is masked. Risk factors associated with presentation of BCS as a consequence of MPN appear to be JAK2V617F positivity, PV, and, paradoxically, for thrombosis risks in general, female sex, and younger age (Table 2).

PV may run a course of uncomplicated disease requiring only phlebotomy for decades, or may follow a progressive, malignant course similar to CML in which a relatively short polycythemic phase is followed by transformation to MF (PPVMF) and then to AML, all occurring within a matter of years from the original PV diagnosis. The PPVMF phase of the disease clinically and molecularly resembles de novo PMF, with high stem cell JAK2 allele burdens and the association of chromosome changes such as 20q deletion, 13q deletion, trisomy 9, and mutation of the ASXL1 gene. The nonrandom chromosomal changes that are present in greater than 60% of patients with PPVMF, and their similar prevalence in de novo PMF, suggests that these lesions drive MF physiology and alter the cellular context of JAK2V617F signaling. Recent studies of PPVMF indicated that approximately 15% of patients developed MF at a median of 10 years after PV diagnosis and that this percentage increased to almost 50% after 20 years. These studies also found that a subset of patients with PV who had lower JAK2V617F allele burdens had a significantly lower risk for this outcome compared with the subset of patients with higher allele burdens (Table 3).

AML occurring directly out of chronic phase PV is unusual and estimated to occur in as many as 4% of patients with PV; whereas, AML evolving from PPVMF is more common, occurring in as many as 20% of patients with MF. The leukemia-accelerating effects of radioactive phosphorus and the alkylator pipobroman on the natural history of PV is uncontested. Moreover, this experience in PV calls any potentially genotoxic therapy into question regarding safety, especially given the long-term exposure to any therapy that some patients with PV will require, and the need for therapy in older patients with PV, where genomic integrity is an issue. Traditional risk factors for AML development include older age, P32 or alkylator therapy, PPVMF, and hemoglobin less than 10 g/dL.

Myelofibrosis

It has long been noted that patients with MF experience markedly different outcomes. To better understand and quantify this phenomenon, a methodical approach to analyzing risk factors associated with higher-risk disease has resulted in the birth of several clinically useful scoring systems that incorporate clinical and laboratory findings in the patient with MF. Cervantes et al examined 1,054 patients with PMF. In this study, the multivariate analysis demonstrated that five risk factors were significantly associated with determination of prognostic score when assessed at time of MF diagnosis: (1) age older than 65 years, (2) leukocytosis (WBC greater than 25 K/uL), (3) circulating blasts of 1% or greater, (4) presence of constitutional symptoms, and (5) anemia of hemoglobin less than 10 g/dL. This divided the patients into groups of low-risk, intermediate-1, intermediate-2, and high-risk, with median overall survivals of 135 months, 95 months, 48 months, and 27 months, respectively. This represents the most widely used scoring system for patients with MF, and, although cytogenetic abnormalities and JAK2V617 mutation were considered, notably, neither reached independent significance to warrant inclusion in the final scoring system in this original International Prognostic Scoring System (IPSS) model. It is of particular importance that the presence of constitutional symptoms were included and indeed validated in the IPSS model for MF, as this highlights an important part of risk stratification for patients with MPNs: the burden of symptoms experienced in these diseases, even in those patients with lower risk scores, affect not only the quality of life, but also has effects on overall survival outcomes.

Several clinical instruments have been developed and validated specifically in patients with MPN, including for patients with MF, to help quantitate and follow these symptom profiles over time. These and other widely available instruments will likely continue to be an important part of recording and understanding MPN’s risks, comorbidities, and symptoms over time, and be a vital method to reliably follow changes/improvements with disease-modifying agents starting to be used in the clinic.

The IPSS scoring method of risk stratification for patients with MF has had a direct effect on therapeutic investigation and decision making as it helped to select an appropriate patient population for the largest randomized clinical trials testing a novel therapy in patients with MF. On the basis of the IPSS, those patients with intermediate-2 or high-risk MF were included on the original phase III clinical trials testing ruxolitinib, a JAK1/2 inhibitor, against either best available therapy or placebo (COMFORT 1 and 2), thus, identifying the highest-risk/poorest-prognosis patients that might benefit from therapy. These trials ultimately led to U.S. Food and Drug Administration approval of ruxolitinib, the first and only therapy specifically approved for patients with MF on the basis of risk stratification, taking into account patient symptom burden and constitutional symptoms into the approval process.

Building on this clinical risk factor model, several other scoring systems for MF have been developed with the aim of providing prognostication for overall survival at any time during disease course, not just at diagnosis, (Dynamic International Prognostic Scoring System [DIPSS]) and further refinement with additional risk factors added (DIPSS-plus), including thrombocytopenia, transfusion dependency, and cytogenetic abnormalities. The identification and success-
ful addition of cytogenetic abnormalities to risk stratification for patients with MF recognizes the striking heterogeneity among patients with this disease at the pathobiologic level, reflects the remarkable strides made in these new widely available laboratory assessments, and is in line with prognostic measurement of other myeloid malignancies (MDS, AML) that are all now utilizing cytogenetic analysis as a part of routine risk stratification and treatment decision making.

Besides cytogenetics, it appears that molecular abnormalities may be crucial to understanding the modern risk stratification of the patient with MF. More recent discoveries of the significance of MPL and CALR mutations added to the already known JAK2 mutation in diagnosis of MF, however, their significance on risk stratification was unknown until recently. Rumi et al examined the effect of molecular mutations on outcomes in 617 patients with MF. The authors found markedly different outcomes based on the molecular profile: the longest median overall survival was found in CALR-mutated (17.7 years), followed by JAK2-mutated (9.2 years), MPL-mutated (9.1 years), and, finally, a group of patients termed triple-negative (negative for CALR, JAK2, and MPL) constituted the poorest-prognosis group with median overall survival of only 3.2 years.

Other genetic lesions important in MDS and AML have been identified at a relatively high frequency in MF and may inform risk. For example, mutations in ASXL1 occur in 30% to 36% of patients with MF. Taking some of these newer markers into account, further molecular risk stratification was conducted with inclusion of and assessment of other molecular mutations commonly found in myeloid malignancies that are also found in patients with MF: IDH, EZH2, SF3B1, SRSF2, U2AF1, and ASXL1. Analyses in large MF cohorts have the power to further refine prognosis based on genetics, with a recent study identifying CALR-negative/ASXL1-positive MF patients exhibiting worse outcomes, suggesting another higher-risk subset.

**CONCLUSION**

In the 10 years since the discovery of the JAK2V617 mutation in MPNs, the field has experienced an exponential increase in terms of clinical and basic science research. With improved methods of stratifying risk for patients at the clinical, biologic, cytogenetic, and, now, molecular level, we are entering a new era of recognizing MPN’s total burden of disease, and we are beginning to consider assigning targeted treatments based on these assessments. The personalization of MPN diagnosis, prognosis, and treatment will likely include the congruence of clinical factors, formal MPN symptom burden assessment, and cytogenetic and molecular analyses.


43. Scott LM, Rebel VI, JAK2 and genomic instability in the myeloproliferative neoplasms: a case of the chicken or the egg? Am J Hematol. 2012;87:1028-1036.


