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Prognostication in Philadelphia Chromosome Negative Myeloproliferative Neoplasms: A review of the recent literature

Amy Zhou, MD, Amber Afzal, MD, and Stephen T. Oh, MD PhD

Division of Hematology, Washington University in St. Louis School of Medicine

Abstract

Purpose of Review—The prognosis for patients with Philadelphia chromosome (Ph)-negative myeloproliferative neoplasms (MPNs) is highly variable. All Ph-negative MPNs carry an increased risk for thrombotic complications, bleeding, and leukemic transformation. Several clinical, biological, and molecular prognostic factors have been identified in recent years which provide important information in guiding management of patients with Ph-negative MPNs. In this review, we critically evaluate the recent published literature and discuss important new developments in clinical and molecular factors that impact survival, disease transformation, and thrombosis in patients with polycythemia vera, essential thrombocythemia, and primary myelofibrosis.

Recent Findings—Recent studies have identified several clinical factors and non-driver mutations to have prognostic impact on Ph-negative MPNs independent of conventional risk stratification and prognostic models. In PV, leukocytosis, abnormal karyotype, phlebotomy requirement on hydroxyurea, increased bone marrow fibrosis, and mutations in *ASXL1*, *SRSF2*, and *IDH2* were identified as additional adverse prognostic factors. In ET, *JAK2* V617F mutation, splenomegaly, and mutations in *SH2B3*, *SF3B1*, *U2AF1*, *TP53*, *IDH2*, and *EZH2* were found to be additional negative prognostic factors. Bone marrow fibrosis and mutations in *ASXL1*, *SRSF2*, *EZH2*, and *IDH1/2* have been found to be additional prognostic factors in PMF. *CALR* mutations appear to be a favorable prognostic factor in PMF, which has not been clearly demonstrated in ET.

Summary—The prognosis for patients with PV, ET, and PMF is dependent upon the presence or absence of several clinical, biological, and molecular risk factors. The significance of additional risk factors identified in these recent studies will need further validation in prospective studies to determine how they may be best utilized in the management of these disorders.

Keywords

Myeloproliferative neoplasms; prognostic factors

Corresponding author: Stephen Oh, M.D., Ph.D., Division of Hematology, Washington University School of Medicine, 660 S. Euclid Ave, Campus Box 8125, St. Louis, MO 63110, Phone: (314) 362-8846, Fax: (314) 362-8826, stoh@wustl.edu.

Conflict of Interest

Amy Zhou, Amber Afzal, and Stephen T. Oh each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent

This article contains no studies with human and animal rights performed by any of the authors.

Introduction

Myeloproliferative neoplasms (MPNs) are a group of myeloid malignancies characterized by clonal proliferation of myeloid lineage cells. The four classic MPNs include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [1]. CML is defined by a reciprocal translocation of chromosome 9 and 22, resulting in the Philadelphia chromosome (Ph), and will not be discussed in this review. Among the Ph-negative MPNs, somatic mutations in Janus kinase 2 (*JAK2*) constitute the most frequent mutation in PV, ET, and PMF [2]. Mutations in calreticulin (*CALR*) and myeloproliferative leukemia virus oncogene (*MPL*) have been identified in patients with ET and PMF who are negative for the *JAK2* mutation [3–7].

The Ph-negative MPNs share similar clinical characteristics such as an increased risk for thrombotic complications, bleeding, and leukemic transformation [8–10]. Patients with PV and ET also have a risk of progression to secondary myelofibrosis (MF), and all three carry a risk of transformation to acute leukemia [11]. The reported prognosis for patients with Ph-negative MPNs is highly variable. Patients with PMF have significantly reduced life expectancy, whereas survival of patients with PV and ET is modestly affected compared to the general population [11, 12]. Clinical and molecular factors have been found to provide additional prognostic information on patients with Ph-negative MPNs, and prognostic models have been developed to further risk stratify patients and guide management [13–17].

In this review, we will critically evaluate the recent published literature on prognostication in Ph-negative MPNs and discuss important new developments in clinical and molecular factors that impact survival, disease transformation, and thrombosis in patients with PV, ET, and PMF.

Prognostic Factors in PV

The prognosis for patients with treated PV is generally favorable, with a median survival of over 13 years. The greatest complication for patients with PV is the risk for venous and arterial thrombosis [18]. Patients with PV are stratified into a high risk or low risk group by the respective presence or absence of age > 60 or prior thrombosis. Survival is moderately decreased compared to that of normal age and sex-matched controls [11, 12], and the most common causes for mortality are cardiovascular events, solid tumors, and hematologic transformation [19].

Clinical prognostic factors

A list of clinical prognostic factors in PV is summarized in Table 1. Age greater than 60 and previous history of thrombosis are the two main predictors of recurrent thrombosis and cardiovascular events [19, 20]. Presence of either one of these two risk factors prompts the recommended addition of cytoreductive therapy to phlebotomy and aspirin [21]. A recent large study of 1545 PV patients from Italy, Austria, and the United States conducted by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT), also found older age and history of venous thrombosis to be predictors of decreased survival. On multivariate analysis, leukocytosis and abnormal karyotype were identified as additional

adverse prognostic factors [22]. A prognostic model that included age, leukocytosis, and venous thrombosis delineated the cohort into three risk groups. Median survival ranged from 27.8 years for the lowest risk group to 10.9 years for the highest risk group. These findings suggest that leukocytosis and abnormal karyotype may be of additional prognostic value, however, the utility of these risk factors in guiding current treatment decisions will need to be evaluated in prospective studies.

The risk of thrombotic complications appears to be significantly reduced when a target hematocrit (Hct) of < 45 percent is achieved [23, 24]. A recent observational study by the Grupo Espanol de Enfermedades Mieloproliferativas Filadelfia Negativas (GEMFIN) investigators of 533 patients from the Spanish Registry of Polycythemia Vera identified phlebotomy requirement on hydroxyurea therapy (defined as three or more phlebotomies per year) as an independent risk factor for thrombosis (HR: 3.3, 95% CI: 1.5 – 6.9; $p = 0.002$) [25]. The study found that patients who required three or more phlebotomies per year in addition to hydroxyurea therapy had significantly higher rates of thrombosis than those who received 0 – 2 phlebotomies per year (20.5% vs. 5.3% at 3 years; $p = 0.002$) [25]. Patients who required three or more phlebotomies per year also showed worse hematocrit control despite requiring higher doses of hydroxyurea. The findings from this study support the results of the CYTO-PV trial [23], suggesting the use of alternative cytoreductive agents in patients who are considered hydroxyurea refractory [26, 27].

Increased bone marrow reticulin fibrosis has been reported with variable frequency in patients with PV [28–31]. In a previous study by the IWG-MRT of 526 PV patients from several centers in Italy, those with increased bone marrow fibrosis (Grade 1) accounted for approximately 14% of patients, and most displayed grade 1 reticulin fibrosis on the initial bone marrow examination [31]. Patients with baseline bone marrow fibrosis were more prone to develop post-PV myelofibrosis during their clinical course and had a higher prevalence of palpable splenomegaly at presentation [31]. There was no significant association between baseline bone marrow fibrosis and overall or leukemia-free survival. A recent study by Barraco et al was performed to validate the prior observations made by the IWG-MRT study and to identify additional risk factors for myelofibrosis-free survival (MFFS) [32]. This study evaluated 262 PV patients from the Mayo Clinic and found that 48% of the patients displayed grade 1 reticulin fibrosis at the time of diagnosis, which is notably higher than the previous study. The discrepancy in the incidence of bone marrow fibrosis may have been related to subjective differences in grading, limiting the ability to directly compare results from the two studies. Nevertheless, similar to the IWG-MRT study, bone marrow fibrosis was associated with decreased MFFS ($P=0.009$) but had no significant impact on overall or leukemia-free survival. Leukocytosis $> 15 \times 10^9/L$, palpable splenomegaly, and grade 1 fibrosis were found to be significantly associated with decreased MFFS on multivariate analysis. This recent study supports the previous findings that bone marrow fibrosis does not impact leukemia free or overall survival in PV patients. Logically, the presence of reticulin fibrosis, leukocytosis, and splenomegaly would decrease MFFS as these are all features of myelofibrosis, and may simply identify patients with secondary myelofibrosis at an earlier course in their disease. As the diagnosis of PV can often be made without a bone marrow biopsy, the utility of performing bone marrow

biopsies to assess fibrosis for prognostic purposes is unclear, as it may not impact treatment decisions and also has not demonstrated an impact on overall survival.

Molecular Prognostic Factors in PV

The majority of patients with PV (>95%) carry a V617F mutation located in exon 14 of the *JAK2* gene. A small percentage of patients who are negative for the *JAK2* V617F mutation harbor a mutation in exon 12 of the *JAK2* gene [33, 34]. Patients with *JAK2* exon 12 mutations do not appear to have a significant difference in their risk of thrombosis, disease transformation, or survival compared to patients with *JAK2* V617F mutation [34, 35]. The development of next generation sequencing (NGS) has allowed the identification of several additional acquired mutations in Ph-negative MPNs, however, these mutations are not restricted to MPNs and are frequently found in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). These additional mutations include DNA methylation regulators (*TET2*, *DNMT3A*, *IDH1/2*), histone modifiers (*EZH2*, *ASXL1*, *IDH1/2*), transcription factors (*TP53*, *CUX1*, *RUNX1*, *ETV6*, *IKZF1*), signaling proteins (*NF1*, *SH2B3/LNK*, *CBL*, *FLT3*, *KRAS*, *NRAS*), and splicing factors (*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*) [36]. Several of these “non-driver” mutations have been shown to have prognostic impact in PMF, but have not been extensively evaluated in PV. A recent study by Tefferi et al, from the Mayo Clinic performed targeted deep sequencing of 27 myeloid neoplasm relevant genes via next generation sequencing of bone marrow or whole blood DNA on a cohort of 133 PV patients and 183 ET patients [37]. Among the 133 PV patients, 53% were found to harbor one or more sequence variants/mutations other than *JAK2*. The most frequent mutations found were in *TET2* and *ASXL1* (Table 2). Mutations in *ASXL1*, *SRSF2*, and *IDH2* were found to be associated with inferior survival in PV patients (median 7.7 vs 16.9 years), independent of conventional prognostic models. These observations were validated in a separate cohort of 215 Italian PV patients [37]. The presence of these adverse variants/mutations was predictive of leukemic and fibrotic progression in both cohorts, although the number of mutations did not provide additional prognostic value, unlike what has been observed in PMF [38]. The findings from this recent study are important in furthering our understanding of the molecular pathogenesis of disease transformation in PV, and should be evaluated in additional cohorts. Whether these additional “non-driver” mutations should be incorporated into current prognostic models (and routine clinical testing) for PV will need further investigation.

Prognostic Factors in ET

ET carries the best prognosis among the Ph-negative MPNs, with most patients having a normal life expectancy [39]. The goal of treatment in ET is to prevent thrombotic and hemorrhagic complications and to alleviate symptoms. Patients with ET are stratified into low risk and high risk groups, similar to PV, based on the respective absence or presence of either age > 60 years or history of thrombosis. Patients who are categorized as high risk are managed with cytoreductive therapy in addition to aspirin therapy [21]. ET patients are at risk for progression to secondary myelofibrosis and acute leukemia, however, the rates of transformation are generally low at about 5 to 10 percent [39–41].

Clinical prognostic factors in ET

In a previous study by the IWG-MRT, advanced age (> 60 years), leukocytosis ($> 11 \times 10^9/L$), and history of thrombosis were identified as risk factors for inferior survival [42]. The presence of cardiovascular risk factors or the *JAK2* V617F mutation were identified as risk factors for thrombosis in a study of 891 ET patients by the IWG-MRT leading to development of the IPSET-thrombosis scoring system to better predict the risk of vascular events [16]. Approximately 60% of patients with ET will have the *JAK2* V617F mutation [43]. The presence of the *JAK2* V617F mutation or cardiovascular risk factors, however, have not led to the routine use of cytoreductive therapy in addition to aspirin in patients who are > 60 years of age and have no history of thrombosis [21]. Recently, further enhancement of the IPSET-thrombosis scoring system was made by reanalysis of the original IPSET-thrombosis data set in order to distinguish therapeutically relevant risk categories that may allow more specific treatment recommendations [17]. The presence of cardiovascular risk factors was not incorporated into the revised IPSET-thrombosis scoring system, which relies on three main variables (thrombosis history, age > 60 years, and *JAK2* V617F mutation) to stratify patients into four risk categories: very low risk, low risk, intermediate risk, and high risk. A recent study done by Haider et al of 585 ET patients from the Mayo Clinic database was performed to validate the revised IPSET-thrombosis scoring system. This study confirmed significant differences in thrombosis risk between “very low” and “low” risk patients (HR 2.4, 95% CI: 1.1 – 5.3) and between “intermediate” and “high” risk patients (HR 2.3, 95% CI: 1.1 – 5.2). On multivariate analysis, only *JAK2* V617F (HR = 1.8, CI: 1.07 – 2.94) and history of thrombosis (HR = 2.1, CI: 1.2 – 3.58) were independently predictive of future thrombotic events [44]. Ultimately, whether cytoreductive therapy should be used in patients with ET who are *JAK2* positive (but with no other risk factors) will need to be confirmed in prospective studies.

Approximately 5 – 25% of ET patients exhibit splenomegaly at diagnosis [16, 45–47]. Previous studies investigating potential risk factors for thrombosis have not specifically addressed the role of spleen enlargement on thrombotic tendency. Recently, Andriani et al performed a retrospective analysis of 1297 patients with ET from 11 Hematologic Centers in Italy in the Lazio Cooperative Group and Bologna University Hospital database evaluating spleen enlargement as a risk factor for thrombosis in ET [48]. Approximately 13% of patients were found to have spleen enlargement. Patients with splenomegaly were generally younger, predominantly male, and presented with higher platelet count and *JAK2* V617F allele burden, and had a lower incidence of concomitant cardiovascular risk factors. Increased thrombotic events were noted in patients who had spleen enlargement versus patients without (15.7% vs 8.6%; $P = 0.003$). Despite comparable use of cytoreductive/antiplatelet therapies in the two groups, the cumulative risk of thrombosis at 5 years was significantly higher in patients with baseline splenomegaly (9.8% versus 4.4%, $P = 0.012$). In multivariate analysis, splenomegaly retained its negative prognostic significance. It is important to note that this study included non-strictly WHO defined ET patients, and therefore splenomegaly may have been an identifier of patients with pre-PMF which are known to have a worse prognosis compared to ET [1, 49]. A recent study by Haider et al of 560 patients from the Mayo Clinic of WHO-defined ET found that patients with splenomegaly developed earlier thrombosis, but the cumulative thrombosis free survival

(TFS) was not significantly different between the two groups [50]. Furthermore, no significant difference was seen between patients with and without splenomegaly in terms of platelet count, hemoglobin level, leukocyte count, prevalence of CV risk factors, or *JAK2* V617F mutational status, unlike the Italian study. The incidence of splenomegaly was higher in the Italian cohort at 13.3% compared to 6.3% in the Mayo Cohort, again highlighting the possibility that patients with pre-PMF were included in the Italian study. Additional studies are needed to clarify the differences seen between these two studies and whether the presence of splenomegaly should impact clinical management.

Molecular prognostic risk factors in ET

Mutations in *JAK2*, *CALR*, and *MPL* are present in approximately 85% of patients with ET at respective frequencies of 58%, 23%, and 4% [13]. The remaining cases are wild type for all three mutations and are termed “triple negative”. More than 80% of *CALR* mutated ET patients harbor one of two mutation variants: type 1, a 52-base pair (bp) deletion (p.L367fs*46) and type 2, a 5-bp insertion (p.K385fs*47), which both lead to a 1+ bp frameshift [3, 4]. In the study by Klampfl et al, a survival advantage for *CALR* mutated patients compared to *JAK2* mutated patients was suggested for both PMF and ET [4]. While subsequent studies have confirmed this in PMF, the results have been conflicting in ET. A recent study by Tefferi et al, of 299 ET patients from the Mayo Clinic did not find a significant difference in survival between *JAK2* mutated and *CALR* mutated ET patients [51]. The study confirmed the association of mutant *CALR* with younger age, higher platelet count, lower hemoglobin, lower leukocyte count, and lower incidence of recurrent thrombosis compared to *JAK2* mutated patients, however, median survival between the two groups was not significantly different (19 years for *JAK2* mutated patients versus 20 years for *CALR* mutated patients; *P* = 0.32).

As mentioned previously, Tefferi et al evaluated the frequency and prognostic impact of additional “non-driver” mutations in patients with PV and ET [37]. Among the 183 ET patients, 53% harbored one or more sequence variants/mutations other than *JAK2/CALR/MPL*. The most frequent mutations found were in *TET2* and *ASXL1* (Table 2). Adverse variants/mutations in ET included *SH2B3*, *SF3B1*, *U2AF1*, *TP53*, *IDH2*, and *EZH2*. Leukemic or fibrotic progression was predicted by the presence of these adverse variants/mutations, although the number of mutations did not provide additional prognostic value. Notably, *ASXL1* and *SRSF2* were not found to be associated with adverse prognosis in the ET cohort, despite conveying a worse outcome in the PV cohort and in PMF [52]. Additionally, *SF3B1* mutations which are associated with favorable prognosis in MDS and MDS/MPN overlap syndromes such as RARS-T [53, 54], were found in this study to be an adverse prognostic factor in ET. This finding is not altogether surprising, as patients with MDS have a considerably overall worse prognosis compared to patients with ET, and presence of this mutation in ET may convey a prognosis that is more similar to MDS.

Prognostic Factors in PMF

Primary myelofibrosis (PMF) carries the worst prognosis among the Ph-negative MPNs [11]. The main causes of death are due to infection and bleeding as a result of progressive

bone marrow failure, thrombosis, and acute leukemia [55]. PMF can evolve to acute leukemia in about 10 – 20% of patients [13, 56]. Decisions for therapy are guided by current prognostic scoring systems such as the International Prognostic Scoring System (IPSS), dynamic IPSS (DIPSS), and DIPSS plus which were developed by the IWG-MRT and are applicable at the time of diagnosis (IPSS) and during the disease course (DIPSS, DIPSS plus) [13–15].

Clinical Prognostic Risk Factors

A list of clinical prognostic factors in PMF is summarized in Table 1. Advanced age, leukocytosis, anemia, transfusion dependence, and elevated inflammatory markers have previously been shown to impact prognosis in patients with PMF [57]. Unfavorable karyotype (complex karyotype, monosomy 7, deletion 7q, trisomy 8, monosomy 5, deletion 5q, deletion 12p, inversion 3, or 11q23) and thrombocytopenia have also been identified as adverse prognostic factors and incorporated into prognostic scoring systems such as the DIPSS and DIPSS plus [15, 58, 59]. The degree of bone marrow fibrosis has not previously been described as a prognostic risk factor and is not incorporated into the current prognostic scoring systems. Recently, a study by Guglielmelli et al evaluated the prognostic impact of bone marrow fibrosis in a cohort of 490 patients with PMF. The study found that fibrosis grade 2 and greater was associated with adverse factors such as anemia, leukopenia, thrombocytopenia, constitutional symptoms, larger splenomegaly, and higher IPSS risk category [60]. Patients with higher grade fibrosis were also more likely to have somatic mutations in *ASXL1* and *EZH2*, which have an adverse effect on survival [38, 52, 61]. In multivariate analysis, fibrosis grade independently predicted for survival regardless of IPSS variables and mutational status, and was noticeable especially in lower IPSS risk categories [60]. A study done by Li et al of 330 patients with PMF in a Chinese cohort also found bone marrow fibrosis grade to be an independent risk factor for overall survival in patients with PMF [62]. Of these patients, 71.2% had bone marrow biopsy at diagnosis and 28.8% were evaluated at the time of referral. The median time between original diagnosis and referral was 36 months (5 – 132 months). The study found that patients with higher grade fibrosis (MF-2 or MF-3) were older, had more frequent hemoglobin concentrations <100 g/l, less frequent WBC levels > 25 × 10⁹/L, more frequent platelet levels <100 × 10⁹/L, higher DIPSS scores, and more frequent unfavorable karyotypes according to DIPSS-plus. Unlike the findings from the Mayo study, however, fibrosis grade was not associated with spleen size or driver mutations. Patients with MF-2 and MF-3 had increased mortality compared to those with MF-0 and MF-1 (31.2% versus 13.1%; P <0.001) at last follow up (median 39 months; 1 – 255 months). The findings from these two studies suggest that bone marrow fibrosis should be incorporated into current prognostic scoring systems, however, the accuracy of fibrosis grading may impact its utility as a prognostic tool.

Molecular prognostic risk factors in PMF

The JAK2 V617F mutation is the most frequent mutation found in PMF (50 – 60%), followed by mutations in *CALR* (25 – 30%) and *MPL* (3–5%). The prognostic impact of the JAK2 V617F mutation has been evaluated in several studies with conflicting results [14, 63–67]. A recent study by Tefferi et al evaluated the prognostic impact of these three driver mutations on a cohort of 254 PMF patients [68]. In univariate analysis, *CALR* mutations

were associated with younger age, higher platelet count, and lower DIPSS-plus score. They were also less likely to be anemic, require transfusions, or display leukocytosis, which are adverse prognostic factors. On multivariate analysis, *CALR* mutations had a favorable impact on survival that was independent of DIPSS-plus risk. Definitive conclusions regarding the impact of *MPL* mutations on prognosis are difficult given the relative low frequency of these mutations (8.3% in this study). The favorable effect of *CALR* mutations in patients with PMF was further supported by another large cohort study by Rumi et al of 617 PMF patients from Italy [71]. A recent meta-analysis by Pei et al of 12 studies involving 435 *CALR* mutated and 1,116 *JAK2* V617F mutated PMF patients found that *CALR*-mutated patients displayed a lower risk of splenomegaly (OR 0.47, 95% CI 0.29 – 0.78) and thrombosis (OR 0.52, 95% CI 0.29 – 0.92), but showed no significant difference in the risk of leukemic transformation (OR 0.90, 95% CI 0.55 – 1.47) when compared to *JAK2*-mutated patients. The presence of *JAK2* V617F was associated with a poorer survival rate compared to *CALR* mutation (HR 2.58, 95% CI 2.08 – 3.20) [69].

Previous studies evaluating the prognostic impact of the two most common *CALR* mutations have been conflicting. A study by Tefferi et al showed that only type-1 or type-1 like *CALR* mutations exerted a favorable prognostic impact, while Type-2 or Type-2 like mutations were associated with survival that was comparable to *JAK2* mutated patients [70]. The study by Rumi et al, however, observed no difference between Type-1 and Type-2 *CALR* mutated PMF patients [71]. A recent study by Li et al on a cohort of 402 PMF patients at a single center in China of 81 *CALR* mutated patients (20.1%) found that patients with Type-2 *CALR* mutations had significantly worse OS compared to patients with Type-1 or Type-1 like *CALR* mutations (P=0.004, HR 6.9, 95% CI 1.9 – 25.4) [72]. Patients with Type-2 *CALR* mutations also appeared to have a worse OS compared to patients with *JAK2* mutations (P=0.046, HR 1.8, 95% CI 1.01 – 3.37) on multivariate analysis. A recent meta-analysis by Kourie et al of 4 studies which included 225 patients with PMF with *CALR* type-1 mutations and 89 with *CALR* type-2 mutations demonstrated a worse prognosis of *CALR* type-2 mutated patients with PMF compared to *CALR* type-1 mutated patients (HR 2.8, 95% CI 1.7 – 4.7) [73]. These recent studies seem to support that *CALR* type-1 and type-2 mutations have different prognostic impact. This may (at least partially) explain why studies in ET have not consistently found a difference in survival between *CALR* and *JAK2* mutated patients, as type-1 *CALR* mutations occur more frequently in PMF [74]. This difference in phenotype between type-1 and type-2 *CALR* mutations has also been demonstrated in mouse models, where type-1 *CALR* mutations were shown to generate a MF-like phenotype in mice and type-2 *CALR* mutations generate an ET-like phenotype [75].

Mutations in *ASXL*, *SRSF2*, *EZH2*, and *IDH1/2* have been found to negatively impact leukemia free survival and OS in PMF [52, 61] (Table 2). The number of these mutations also appeared to correlate with OS and LFS in patients with PMF, with patients harboring two or more of the above identified mutations having a worse OS and shortened LFS compared to patients harboring only one mutation [38]. Mutations in *U2AF1* have previously been reported to be strongly associated with anemia and thrombocytopenia, both of which have been validated as negative clinical prognostic markers [76]. A recent study by Barraco et al further evaluated the correlation between anemia and PMF in 722 PMF patients from the Mayo Clinic and found mutations in *U2AF1* to be associated with inferior survival

compared to unmutated patients (median survival 3.75 years versus 5.74 years, $P > 0.0001$), however, this significance was lost when analysis was adjusted for age and anemia ($P = 0.24$) [77]. The authors concluded that screening for *U2AF1* in MPF patients may be needed to identify those who are at risk for development of anemia, however, this study did not demonstrate that *U2AF1* is predictive of anemia so much as it is associated with it. Although these findings provide important insight into the disease pathogenesis of these molecular mutations, routine screening for mutations in *U2AF1* is of questionable clinical utility at this time.

Conclusion

The prognosis for patients with PV, ET, and PMF is modified by the presence or absence of several clinical, biological, and molecular risk factors. The clinical course for each of these diseases is highly variable, and our understanding of the pathogenesis of these disease is evolving as new discoveries are made. While the prognosis for patients with PV and ET are generally favorable, the outcomes for patients with PMF remain dismal. The significance of additional risk factors identified in these recent studies will need further validation in prospective studies to determine how they may be best utilized in the management of these disorders.

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Table 1

Summary of adverse clinical prognostic factors in PV, ET, and PMF

PV	ET	PMF
Age > 60	Age > 60	Age > 65
Prior history of thrombosis	Prior history of thrombosis	Hemoglobin < 10 g/dL
Leukocytosis (WBC $\geq 15 \times 10^9/L$)	Leukocytosis (WBC $\geq 11 \times 10^9/L$)	Leukocyte count > 25,000/microL
Abnormal karyotype	Splenomegaly	Circulating blast cells ≥ 1 percent
Increased bone marrow fibrosis (Grade ≥ 1)		Presence of constitutional symptoms
Splenomegaly		Unfavorable karyotype *
		Platelet count < 100,000/microL
		Transfusion need
		Bone marrow fibrosis grade ≥ 2

* Unfavorable karyotype includes: Complex karyotype or one or two abnormalities that include +8, -7/7q-, -5/5q-, 12p-, inv(3), or 11q23

Table 2

Mutational frequencies in PV, ET, and PMF

Gene	PV	ET	PMF
<i>JAK2</i>	> 95% V617F, 3% exon 12 mutations	50 – 60%	50 – 60%
<i>CALR</i>	Infrequent	25 – 30%	25 – 30%
<i>MPL</i>	Infrequent	3 – 5%	3 – 5%
<i>ASXL1</i>	12% ¹	11% ¹	21.7% ²
<i>TET2</i>	22% ¹	16% ¹	9.7% ²
<i>SRSF2</i>	3% ¹	2% ¹	8.5% ²
<i>IDH2</i>	2% ¹	1% ¹	2.6% ²
<i>SH2B3/LNK</i>	9% ¹	3% ¹	Infrequent ⁴
<i>SF3B1</i>	3% ¹	5% ¹	8% ³
<i>U2AF1</i>	0% ¹	1% ¹	16% ³
<i>TP53</i>	1% ¹	2% ¹	4% ⁴
<i>EZH2</i>	0% ¹	3% ¹	5.1% ²
<i>IDH1</i>	0% ¹	1% ¹	2.6% ²

¹Frequencies are from Tefferi et al. Targeted deep sequencing in polycythemia vera and essential thrombocythemia. Blood Advances 2016. 1 (1): p. 21–30.

²Frequencies are from Vannucchi et al. Mutations and prognosis in primary myelofibrosis. Leukemia 2013. 27(9):p. 1861–9.

³Frequencies are from Barraco et al. Molecular correlates of anemia in primary myelofibrosis: a significant and independent association with U2AF1 mutations. Blood Cancer J 2016. 6:p.e416.

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