Risk Factors for Disease Progression and Treatment Goals in Polycythemia Vera

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Abstract: Polycythemia vera is a Philadelphia chromosome–negative myeloproliferative neoplasm characterized by the clonal proliferation of hematopoietic cells, leading to the overproduction of erythrocytes and the elaboration of inflammatory cytokines. Management is aimed at reducing the risk of thromboembolic events, alleviating the symptom burden, decreasing splenomegaly, and potentially mitigating the risk of disease progression. Existing treatment options include therapeutic phlebotomy and cytoreductive agents including hydroxyurea, pegylated recombinant interferon alpha 2a, ropegylated recombinant interferon alpha 2b, and ruxolitinib. We review risk factors for both thrombotic events and disease progression in patients with polycythemia vera. We discuss existing and novel therapeutic approaches to mitigate the risk of disease-related complications and progression.

Keywords
Debated practices, disease progression, myeloproliferative neoplasm, polycythemia vera

Introduction

Polycythemia vera (PV) is one of the Philadelphia chromosome–negative myeloproliferative neoplasms (MPNs), which include essential thrombocythemia (ET), PV, and myelofibrosis (MF). PV is characterized by an autonomous overproduction of red blood cells (RBCs) and has an estimated incidence of 2.8 cases per 100,000 patients per year.[1] PV is driven by an activating mutation in the JAK-STAT pathway, usually a *JAK2* V617F mutation but sometimes a *JAK2* exon 12 mutation.[2,3] Activation of this cellular signaling cascade results in erythropoietin (EPO)-independent clonal proliferation of erythroid precursors, increasing red cell mass (RCM), extramedullary hematopoiesis, and inflammatory cytokine production.[4] *JAK2*-mutated hematopoietic progenitor and stem cells (HPSCs) secrete IL1β, TNFα, IL-12, and IFNγ, among other proinflammatory cytokines, which provide a survival advantage to the mutated HPSCs and serve as promoters of mutagenesis.[5-9] In turn, patients experience a constellation of clinical complications, which include an increased risk of thrombosis and hemorrhagic events, hepatosplenomegaly due to extramedullary hematopoiesis, constitutional and microvascular symptom burden, and proclivity to progress to MF and/or an aggressive form of acute

leukemia known as MPN-blast phase (MPN-BP).[4,10] In addition to causing overproliferation of erythrocytes, the inflammatory cytokine milieu drives the production of other myeloid cell lines, often leading to concurrent thrombocytosis and leukocytosis.[11]

Most of the disease-related morbidity is related to the increased risk of arterial and venous thrombotic events and the focus of therapeutic development in PV was aimed at mitigating this risk.[12] PV is a progressive disease by nature. Progression to MF is common, with 20-year rates ranging from 11% to 45%.[13] Progression to MPN-BP is much rarer, occurring at a rate of 2% to 4%.[14-16] Of the available therapeutic agents, the JAK1/2 inhibitor ruxolitinib (Jakafi, Incyte) and recombinant pegylated interferon (pegrIFN) alpha-2a and -2b have demonstrated the ability to target and eliminate the underlying malignant clone. Data suggest that this correlates with clinical benefit in terms of decreased risk of disease progression and overall survival (OS) benefit, but there has yet to be a causal relationship established.[17,18] Management largely remains focused on reducing the risk of thromboembolic events and improving disease-related symptoms and splenomegaly, but there is great interest in developing and implementing therapies that have the potential to alter the progressive nature of the disease. In this review, we discuss factors that contribute to the risk of disease-related complications, including thrombotic events and disease progression, and discuss current management to mitigate these risks.

Diagnostic and Response Criteria

The diagnosis of PV is based on the 2022 World Health Organization (WHO) revised criteria[19] or the 2022 International Consensus Classification (ICC) diagnostic guidelines.[20] These diagnostic tools differ from each other in that the ICC includes RCM and requires a *JAK2* mutation rather than characteristic histopathologic changes to meet diagnostic criteria as required by the WHO 2022 criteria (Table). Although there is no definitive consensus among experts regarding which of these consensus criteria is optimal, it is vital to understand the implications of their differences.

The ICC criteria allow for bypassing bone marrow examination in *JAK2*-mutated cases with elevated hematocrit (HCT). Although we implement this into our clinical practice to optimize risk stratification by establishing the level of baseline fibrosis, evaluating cytogenetics, and performing next-generation sequencing (NGS), fewer than 25% of patients undergo a bone marrow biopsy at the time of diagnosis.[21] The WHO does not require confirmation of a *JAK2* mutation for diagnosis in those with elevated HCT or elevated RCM with characteristic histopathologic findings suggestive of PV. Although there are case reports of *JAK2*-unmutated PV, this is exceedingly rare and is more often a process consistent with another disease entity in our experience.

Both the ICC and WHO allow for elevations in HCT as a key diagnostic criterion. The ICC also allows RCM to be considered, but this is not a requirement as the criteria are written. RCM is largely a forgone measurement owing to the logistical challenges and expense of this test. This test provides a true measurement of RCM and plasma volume to avoid issues of hemoconcentration or hemodilution, additionally taking into account the mean corpuscular volume, which can be influenced by therapies such as hydroxyurea (HU). Unfortunately, this test is no longer widely accessible because it is useful in determining the extent of absolute erythrocytosis.

Despite recent updates in diagnostic criteria, there have not been updated response criteria outlined since 2013. A complete response (CR) to therapy is defined by the 2013 International Working Group–Myeloproliferative Neoplasm Research and Treatment (IWG-MRT) and European LeukemiaNet (ELN) as the meeting of all the following criteria: (1) resolution of disease-related symptoms and splenomegaly; (2) absence of hemorrhagic or thrombotic events; (3) no evidence of progression of disease; (4) normalization of bone marrow histology; and (5) normalization of peripheral complete blood count, including hemoglobin and HCT as well as white blood cell (WBC) and platelet count.[22]

Risk Stratification

Age and thrombotic history remain the only 2 factors included in clinically implemented risk stratification tools for patients with PV. Those older than 60 years and/or with a history of venous or arterial thrombosis are included in the most up-to-date 2011 ELN high-risk category.[23] This stems primarily from a study conducted by the IWG-MRT that evaluated 1545 patients with PV and found that prior arterial events were predictors of subsequent arterial thrombosis, and prior venous events and being older than 65 years predicted venous thrombosis.[24,25]

Although age older than 60 years and thrombotic history are the 2 key factors that are clinically implemented in current risk stratification scores, numerous other prognostic factors must be considered when managing patients with PV.

Splenomegaly

Baseline splenomegaly, defined as either palpable splenomegaly greater than 5 cm below the left costal margin or the emergence of newly palpable splenomegaly, is a risk

factor for fibrotic transformation.[26] Splenomegaly has been associated with an increased risk of fibrotic transformation and leukemic evolution, as well as reduced OS. One study found that in patients with PV, thrombosis or cardiovascular events occurred in 44.44% of patients with splenomegaly and in 30.39% of patients without splenomegaly (P=.02).[27] A recent single-institution retrospective study showed an association between splenomegaly at diagnosis or presentation and an increased risk of progression to MF, independent of age or disease duration.[28]

Erythrocytosis

Because thrombotic events contribute most to disease-related mortality, the primary goal of PV management is to minimize the risk of such events. Along with low-dose aspirin, which is recommended for all patients with PV, the cornerstone of management includes maintaining the HCT level below 45% using therapeutic phlebotomy; a variety of cytoreductive therapies including HU, pegrIFNα-2a or recombinant ropegylated interferon alpha 2b (ropegrIFNα-2b; Besremi, PharmaEssentia); or ruxolitinib. Although the pathophysiology of thrombosis is complex and involves multiple patient- and disease-related variables, it is thought that the high burden of erythrocytosis contributes to thrombotic risk by increasing blood viscosity.[29,30] This viscosity displaces platelets toward vessel walls, leading to increased platelet interaction with endothelium and resulting in endothelial damage and upregulation of soluble procoagulant factors.[31] Accordingly, it is logical to hypothesize that normalizing HCT will improve this biological cascade and reduce the risk of thrombosis.

The HCT target of less than 45%, which is included in the 2013 IWG-MRT response criteria, was established in 1978 by Pearson and colleagues[32] and later reaffirmed by the CYTO-PV trial, which randomized patients to a strict (<45%) or liberal (45%-50%) HCT goal. In addition to phlebotomy, clinicians had the option to use cytoreductive agents of choice. There was a 4-fold risk reduction in death by cardiovascular cause or thrombotic events in those who were randomized to the strict HCT goal (2.7% vs 9.8%; P=.0007).[33] This confirmed the recommendation to maintain HCT below 45% with phlebotomy or the addition of cytoreduction in high-risk PV patients. Although there are no prospective data to support lower HCT targets, some clinicians have implemented an HCT goal below 42% for female patients because women have lower RCM and an increased risk of hepatic vein thrombosis compared with men after controlling for HCT levels.[34,35] This practice is neither guideline-directed nor included within the 2013 IWG-MRT response criteria.

To achieve HCT control, phlebotomy is the current first-line therapy for patients with low-risk PV by ELN criteria.[36] Administering cytoreductive agents in low-risk PV is recommended in certain patient populations, particularly those with symptomatic splenomegaly or high disease-related symptom burden,[37] persistent leukocytosis, extreme thrombocytosis, or inadequate HCT control despite phlebotomy.[38] A cytoreductive agent is recommended as a first-line treatment option in those with ELN high-risk PV unless contraindications exist.[39,40]

Leukocytosis

Owing to the proinflammatory milieu, patients with PV frequently present with leukocytosis and thrombocytosis.[11] As discussed, normalization of hematologic parameters—including WBC count and platelet count—is required to achieve a CR by 2013 ELN/IWG-MRT response criteria.[22] At present, the treatment goal of PV is to effectively manage symptom burden and splenomegaly and to minimize the risk of thrombotic events and progression of disease. To accomplish these goals, we consider whether baseline leukocyte count or correction of leukocytosis has prognostic implications.

Leukocytosis at the time of diagnosis has been associated with adverse prognostic implications, including the risk of thrombotic events, progression of disease, and reduced OS.[16,41,42] Activated leukocytes express pro-coagulant factors, including tissue factors and cytokines, including IL1β and TNFα, which contribute to the risk of thrombosis and promote fibrotic changes within the bone marrow.[43]

Despite the association of leukocytosis with the risk of thrombosis and disease progression, much of the literature does not use repeated measures to predict whether a change in leukocytosis, or resolution therein, mitigates the risk of thrombosis or transformation of disease.[44] Our group has previously shown that the leukocytosis trajectory is associated with an increased risk of disease evolution, but not thrombotic events. This retrospective evaluation of more than 500 PV patients from multiple US centers grouped patients into leukocyte trajectories with stable WBC levels at low, intermediate, borderline high, and markedly elevated counts.[45] These data further supports leukocytosis as a dynamic variable that holds prognostic impact; however, they do not inform whether normalization of WBC count mitigates the risk of disease progression or thrombotic events.

Subsequently, our group retrospectively evaluated the outcomes of 527 patients with PV to determine whether ELN response predicts thrombotic events or death using multivariable Cox proportional hazard methods.[46] There was no association between achieving CR by ELN response criteria and the risk of thrombosis or death. However, those who achieved ELN responses had a decreased risk of disease progression to MF without a decreased risk of thrombosis or death.[47] These data suggest that the current ELN response criteria are not

informative when evaluating new therapeutic strategies with the primary goal of mitigating thrombotic risk or preventing disease-related death, but may be valuable in determining the risk of disease progression.

There are conflicting data that suggest the prognostic value of normalized hematologic parameters, including leukocytosis. The outcomes of a group of 261 patients with PV who were treated with HU for a median of 4.4 years were evaluated retrospectively. The median time of follow-up was 7.8 years.[48] The authors reported that when each of the criteria included within the composite ELN response were individually assessed by multivariate analysis, only normalization of the WBC count had prognostic implications. In this study, there was no prognostic value associated with a lack of response in HCT level, platelet count, symptoms, or spleen size. Those who did not achieve a leukocyte response had an increased risk of all-cause death (hazard ratio [HR], 2.7; *P*=.007) and those who did not sustain a WBC response had an increased risk of hematologic transformation to MF or MPN-BP (HR, 3.2; *P*=.004).[48] This suggests a potential benefit in leukocyte control in terms of mitigating the risk of transformation and prolonging OS.

Subsequently, the impact of leukocytosis on thrombotic risk was evaluated in a subanalysis of the CYTO-PV study. Investigators found that patients randomized to the less-strict HCT goal (45%-50%) had higher leukocyte counts than those treated with the strict HCT goal (<45%).[49] This study constructed a multivariable, time-dependent hazard model that stratified patients by leukocyte count at the clinic visit preceding a thrombotic event. They demonstrated that there was a statistically significant increased risk of a thrombotic event with a WBC count of at least 11 × 10^9/L.[49] This type of modeling, however, is limited in that it does not establish a true causal relationship between leukocytosis and thrombotic risk. It does demonstrate that before the detection of a thrombotic event, patients are more likely to have leukocytosis (WBC >11 × 10^9/L). Additionally, this study does not establish whether normalization of leukocytosis using cytoreductive therapies correlates with a reduced thrombotic risk. Given conflicting retrospective data and a lack of prospective data regarding the impact of leukocyte count control in PV, this remains an area of interest requiring further investigation.

Thrombocytosis

Because platelets are necessary for the formation of thrombi, there has been significant interest in understanding whether normalization of elevated platelet counts (>400 × 10^9/L) leads to a reduction in the risk of thrombotic events. Increasing platelet counts has been shown to increase the blood viscosity in a mouse model of PV and lead to decreased cerebral capillary flow, so platelet counts have been postulated to play a role in thrombotic and microvascular complications.[50] However, across multiple large studies evaluating risk factors for thrombosis and progression of disease, there has been no association of either venous or arterial thrombotic events or disease progression with elevated platelet counts.[41,45,51-55] In fact, platelet counts greater than 1000 × 10^9/L are associated with a decreased risk of arterial thrombotic events and an increased risk of hemorrhagic events due to the development of acquired Von Willebrand syndrome (aVWS). Large quantities of circulating platelets bind to Von Willebrand Factor (VWF), leading to adsorption and increased clearance of VWF multimers.[56] Therefore, with an increased risk of hemorrhagic events, individuals with severe thrombocytosis should be screened for aVWS. If there is evidence of this process, cytoreduction should be considered to minimize this risk. However, because no data suggest that achieving a platelet count below 400 × 10^9/L alters the risk of thrombotic events, affects symptom burden, or changes the trajectory of disease, the criteria that necessitate normalization of platelet count to achieve a CR should be critically evaluated. The recommendation to attempt to achieve hematologic CR may lead to overtreatment, with potential unnecessary toxicity.

Bone Marrow Biopsy Characteristics

Although performing a bone marrow biopsy is not necessary to establish a diagnosis of PV, histopathologic findings may provide prognostic insight into the risk of disease progression. Baseline bone marrow fibrosis, present in about 14% to 48% of patients at presentation, has been shown across multiple studies to be independently associated with an increased risk of disease progression to post-PV MF.[57,58] Although one study suggested that baseline fibrosis is associated with a decreased risk of thrombosis, the relatively rare occurrence of both fibrosis and thrombotic events makes these findings questionable. Further studies will be required to better elucidate a relationship. Given the impact of histopathologic findings on prognosis, it is important to offer and discuss the potential value of obtaining a baseline bone marrow biopsy in patients with newly diagnosed PV to better inform the risk of disease progression.

Cytogenetics

A lack of cytogenetic testing at the time of diagnosis, compounded by a relatively low frequency of abnormal karyotypes, has historically posed challenges in integrating cytogenetics in PV prognostication tools. A study conducted by the IWG-MRT that evaluated 1545 patients with PV found that those with an abnormal karyotype have a higher risk of disease progression; however, the number of patients with cytogenetic abnormalities was

too small to determine the prognosis or the impact of individual cytogenetic abnormalities.[24] Having an abnormal karyotype at diagnosis was documented in 12% of the 631 patients examined. In the largest retrospective study to date, 422 PV patients with baseline cytogenetic data were evaluated. Those with an abnormal karyotype at the time of diagnosis demonstrated a higher risk of disease progression and a shorter period of transformation-free survival compared with those with a normal karyotype. This study risk-stratified cytogenetic information into 3 risk groups—low (normal karyotype, sole +8, +9 and other single abnormality), intermediate (del[20q], or double abnormalities), and high (complex karyotype)—and demonstrated significant prognostic implications in terms of OS among these groups. The associated median OS was found to be 137 months, 129 months, and unreached, respectively. In those with del(20q), or double karyotypic abnormalities, the median OS was found to be 86 months and 91 months, respectively. In those with complex karyotypes, the median OS was 9 months.[59] Accordingly, it is likely that cytogenetic abnormalities have significant prognostic implications for disease progression. However, owing to the relative rarity of PV and the infrequency of cytogenetic abnormalities and testing alike at the time of diagnosis, it remains challenging to integrate cytogenetics into clinically meaningful prognostic tools. Prospective studies with larger patient populations will need to be conducted to better understand the full prognostic implications of cytogenetic abnormalities in PV.

Genomic Risk Factors

The *JAK2* V617F variant is the most frequent driver mutation in *BCR-ABL1*–negative MPNs, detected in 97% of PV cases.[53] The impact of the *JAK2* V617F allelic burden and the presence of additional somatic mutations that may occur within the malignant clone on the development of thromboembolic events, leukemic progression, and OS has been evaluated across several studies. A correlation has been described between a high variant allelic frequency (VAF) of mutated *JAK2* and clinical presentation, including pruritus, myelopoiesis, and splenomegaly (all positive), and the platelet count (negative).[60]

A high *JAK2* V617F allele burden has also been associated with an increased risk of thrombotic events and disease progression. In one study evaluating PV patients, *JAK2* V617F VAF of greater than 50% was identified as a risk factor for venous thrombosis independent of conventional risk stratification. However, in this study, *JAK2* V617F VAF of greater than 50% was not shown to be a risk factor for arterial thrombosis.[61] Based on these findings, high VAF is considered and may be incorporated as an independent adverse prognostic variable into our baseline risk stratification tools.

The impact of reducing *JAK2* V617F VAF on disease trajectory remains unknown. In theory, reducing or eliminating the detectability of the underlying malignant clone through mutational surrogacy may have the potential to slow the progression of disease and minimize disease-related complications; however, this has yet to be fully validated in prospective studies. Until recently, of the therapeutic agents used to treat PV, only pegrIFNα-2a and ropegrIFNα-2b had been shown to effectively target the underlying malignant clone and lead to molecular remissions, or even elimination of detectable driver mutation. Again, whether this leads to a meaningful difference in clinical outcomes such as progression-free survival and OS remains suggested but not completely elucidated. Across 3 clinical trials that have evaluated pegrIFNα-2a or ropegIFNα-2b as therapeutic agents in PV, molecular responses, defined as a reduction in driver VAF of at least 50%, ranged from 54% to 72% in patients evaluated. Complete molecular response, defined as a 100% reduction in driver VAF, occurred in 14% to 24% of patients, suggesting the ability of these agents to fundamentally target the MPN stem and progenitor cell population.[62,63]

Molecular responses are not included in the 2013 IWG-MRT response criteria; however, as we gain more prospective evidence confirming the clinical implications of these molecular responses, we believe they should be considered by regulatory agencies as surrogate endpoints and more closely evaluated in future guidelines, as this may have important clinical management implications for our patients.

NGS has identified multiple somatic mutations that may significantly change the diagnostic and prognostic assessment of patients with PV. The pathogenetic role of acquired somatic mutations and their impact on disease progression and leukemic transformation is actively being explored. Several of these genes have been implicated in the progression to MF and AML.[64] Genetic mutations affecting epigenetic regulatory mechanisms, specifically DNA methylation and chromatin modification genes, including *TET2,* isocitrate dehydrogenase 1/2 (*IDH1/2*), and *ASXL1*, are described as more frequent in PV than in ET. *TP53* mutations have also been associated with overall poor prognosis.[64-66]

Loss of TET2 function leads to dysregulated gene expression in hematopoietic stem cells and has been considered a potential initiation step of myeloid and lymphoid malignant transformation in mice. It has been reported that *TET2* mutations are associated with nearly 20% of cases.[67,68] However, there are no data at this time that implicates TET2 as prognostically significant in PV. Therefore, the implications of *TET2* mutations in terms of disease progression risk require further exploration.

ASXL1 is responsible for maintaining the activation

and silencing of proteins that regulate developmental genes by binding and regulating chromatin structure. It is known to be a driver mutation in clonal hematopoiesis and myeloid malignancies and is associated with poor outcomes in myelodysplastic syndrome and acute myeloid leukemia. Disruption of this gene is identified in only 4% to 7% of cases of PV.[65] The role of *ASXL1* in normal hematopoiesis remains poorly understood, but mutations contribute to fibrotic progression. Within the context of PMF, *ASXL1* mutations are common (19%-40%) and are associated with poor survival.[69] Numerous studies have demonstrated an increased risk of thrombotic events and disease transformation as well as decreased OS in *ASXL1*-mutated PV.[18,70-72]

IDH1 and *IDH2* mutations confer neoenzymatic activity of mutant *IDH1/2*, leading to the accumulation of oncometabolite 2-hydroxyglutarate (2-HG), and are associated with numerous myeloid malignancies.[73] They are present in approximately 2% of PV cases with no known adverse effect on survival; however, any prognostic impact of this mutation would be challenging to detect based on the rare incidence.[74] Although *IDH1* and *IDH2* mutations are targetable in more aggressive myeloid malignancies, including AML and myelodysplastic syndrome, the role of targeting IDH1 and IDH2 in PV is unknown. Owing to the relative rarity of these mutations and the potential treatment-related toxicities associated with these inhibitors, they are not currently in clinical testing.

TP53, a 'master regulator' of a diverse array of cellular processes, codes for tumor suppressor protein p53. Aberrations of this gene play a critical role in the pathogenesis of numerous malignancies. *TP53* mutations occur in 6% to 8% of PV patients, but in as many as 66% of patients with MPN-accelerated or MPN-BP.[75,76] Acquiring *TP53* mutations is associated with disease progression and an overall poor prognosis. There have been multiple attempts to target *TP53* mutations in AML and MDS. The most notable therapeutic candidate, APR-246, demonstrated encouraging preclinical and early-phase clinical data in its ability to increase p53 activity in mutated *TP53* cell lines. However, in a later-phase trial, this did not translate into clinical benefit in patients with AML/MDS.

Therapeutic Interventions

Aspirin

Low-dose aspirin is implemented into the treatment regimen in nearly all patients with PV unless there is a contraindication or the patient is receiving anticoagulation for a prior thrombotic event or comorbid condition. The efficacy and safety of aspirin were evaluated in the ECLAP randomized clinical trial, which demonstrated a significant reduction in the combined endpoint of cardiovascular death, nonfatal myocardial infarction, stroke, and major venous thromboembolism, without an increased risk of hemorrhagic events. This study was limited in that patients were not stratified by disease risk or cardiovascular risk, and there was overall poor enrollment. Additionally, enrolled patients also had poor HCT control, limiting interpretation.[77] Nonetheless, implementation of low-dose aspirin is recommended in all patients with PV unless contraindicated, given the likely benefit, despite the weaknesses of prior studies.

Hydroxyurea

HU is a frequently used first-line therapy option for cytoreduction in high-risk PV (see Figure). The PVSG protocol 08 was a nonrandomized observational study that demonstrated a reduced risk of thrombotic events in 51 patients treated with HU vs 131 historic controls at 2-year follow-up (10% vs 33%).[39] Patients in the historical control arm had poorly controlled HCT, increasing the risk of thrombosis and potentially introducing bias that would overestimate the benefit of HU. The consensus from ELN and NCCN supports the use of HU in high-risk cases as a first-line therapy option in high-risk PV.[38] Patients who are at intermediate risk (>60 years without prior thrombosis) may benefit from HU, but require shared decision-making to determine whether to initiate HU. Patients can eventually become resistant or experience intolerable side effects (eg, skin ulcers, gastrointestinal problems, oral ulcers, and nonmelanoma skin malignancies); however, these are rare complications and HU is generally well-tolerated.[78] Unlike rIFNα, HU has no documented impact on targeting the underlying malignant clone, and therefore has minimal, if any, impact on modulating the risk of disease progression.

The response, or lack thereof, to HU can provide meaningful prognostic information. A study that evaluated the outcomes of 216 PV patients demonstrated that resistance to HU, which occurred in 10% of patients included in the study, increased the risk of death (HR, 5.6; 95% CI, 2.7%-11.9%) and transformation of disease (HR, 6.8; 95% CI, 3%-15.4%) compared with those who achieved a complete or partial response on HU.[48] These poorer outcomes must be considered when evaluating clinical trials that include HU resistant/refractory PV.

Recombinant Interferon Alpha

Recombinant IFNα and pegrIFNα-2a have been used off-label for decades to effectively reduce and normalize HCT and decrease the disease-associated risk of thrombosis. There is a growing body of evidence demonstrating the ability of these agents to target the underlying malignant clone and reduce *JAK2* V617F VAF as a surrogate marker of disease burden. RopegrIFNα-2b is a monopegylated

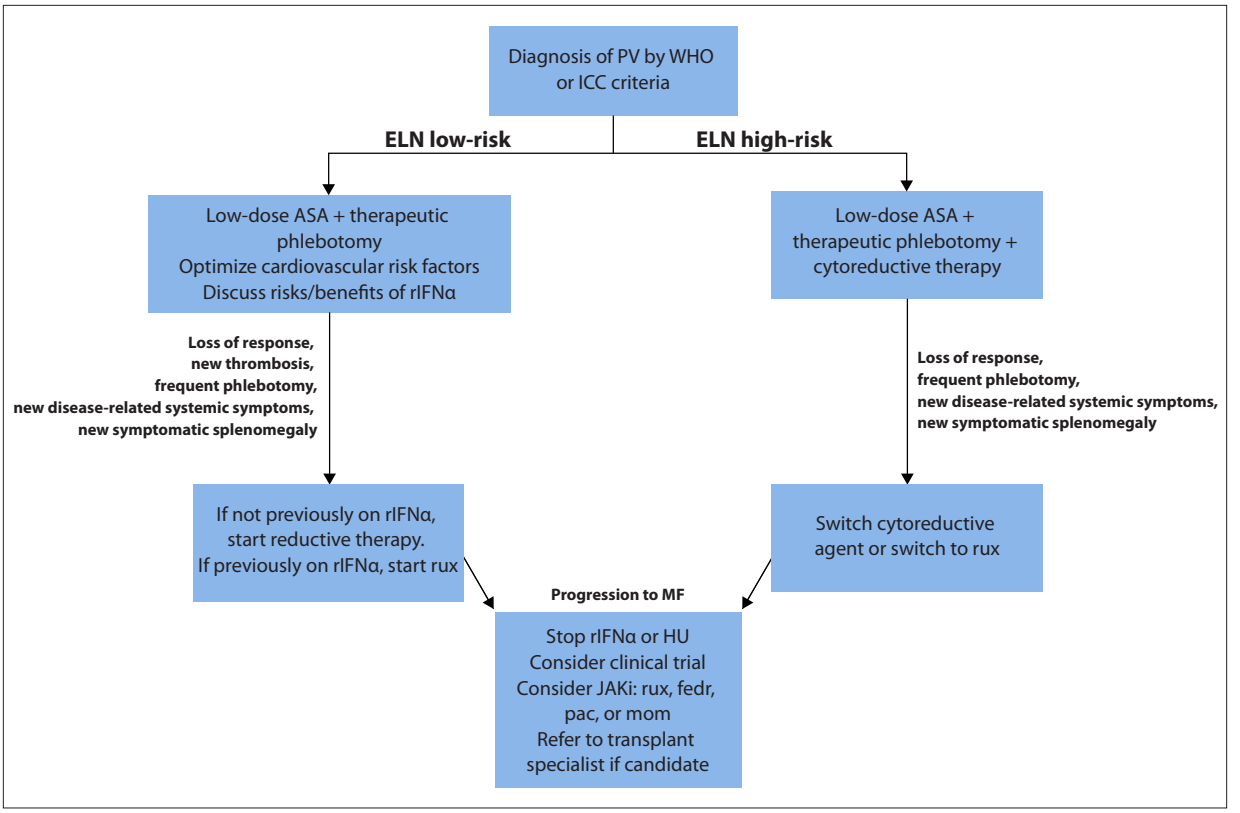

Figure. Treatment schema of polycythemia vera.

ASA, aspirin; ELN, European LeukemiaNet; fedr, fedratinib; HU, hydroxyurea; ICC, International Consensus Classification; JAKi, Janus kinase inhibitor; MF, myelofibrosis; mom, momelotinib; pac, pacritinib; PV, polycythemia vera; rIFNα, recombinant pegylated interferon alpha 2a or recombinant ropegylated interferon alpha 2b; rux, ruxolitinib; WHO, World Health Organization.

formulation that has an extended half-life, imparting an improved toxicity profile and reduced-frequency dosing (every 2 weeks). RopegrIFNα-2b was approved by the US Food and Drug Administration (FDA) for the treatment of PV in the frontline or refractory setting in November 2021.[79]

There have been 3 clinically relevant phase 2 clinical trials that have evaluated pegrIFNα-2a. Together, these trials demonstrated tolerability and overall response rates (ORRs) ranging from 80% to 95% when used in the frontline setting, and 60% in the HU-refractory or -intolerant setting.[62,63,80] HCT control, defined as an HCT level below 45% in the absence of therapeutic phlebotomy, occurred in 46% to 95% of patients enrolled in these trials. Additionally, 37% to 97% of phlebotomy-dependent patients at baseline achieved phlebotomy independence after 1 year of treatment with pegrIFNα-2a.[62,63,80]

The optimal frontline cytoreductive agent in ELN high-risk PV remains debated. In the phase 3 MPN-RC trial that randomized patients with PV to receive either HU or pegrIFNα-2a, there was no significant difference in the CR rate at 12 months; however, there were more frequent severe (grade ≥3) adverse events (AEs) in those treated with pegrIFNα-2a.[81] It should be noted that the primary endpoint in this trial was at 12 months, which is likely too short a time to recognize any potential differences in efficacy between HU and pegrIFNα-2a.

Development of ropegrIFNα-2b led to the phase 3 PROUD/CONTINUATION-PV studies, which randomized patients with early-stage PV (no history of cytoreductive therapy, or a less than 3-year history of previous HU therapy) to receive either HU or the novel monopegylated form of rIFNα2b. After 36 months of therapy, more patients treated with the ropegrIFNα-2b achieved complete hematologic remission (normalization of complete blood count without phlebotomy) and normalization of spleen size compared with those who were treated with HU (53% vs 35%). The difference in response rate became more apparent after 12 months of therapy. Additionally, more patients treated with ropegrIFNα-2b achieved a molecular response than those treated with HU (66% vs 27%). Notable toxicities of rIFNα include flu-like symptoms and neuropsychiatric effects. These should be discussed with patients, particularly those with a history of

psychiatric comorbidities.[17] The results of the PROUD/Continuation-PV study led to the approval of ropegrIFNα-2b for frontline use in PV in the United States and Europe. Despite a worse toxicity profile compared with HU, the use of pegrIFNα-2a or ropegrIFNα-2b in the frontline setting has become increasingly more attractive with the recent FDA approval of a ropegrIFNα-2b and a growing body of evidence that supports the disease-modifying potential of this agent. A decrease in the measured driver mutation *JAK2* V617F VAF as demonstrated across multiple preclinical and clinical studies may provide clinical benefit and perhaps even alter the disease trajectory; however, there is a dearth of prospective data to date that demonstrate direct clinical benefit in terms of reduced thrombotic risk or disease progression.

Whereas the MPN-RC and PROUD/CONTINUATION-PV study evaluated patients with high-risk PV, the LOW-PV study demonstrated that ropegrIFNα-2b can be safely administered in patients with low-risk PV, and is more effective than phlebotomy alone at maintaining HCT below 45%.[37] There was only one thrombotic event in the standard therapy arm and no thrombotic events in the experimental arm. Given the low frequency of thrombotic events in the limited follow-up time of only 24 months, it is impossible to determine whether there is a significant impact on thrombotic risk in this patient population. There was a significant decrease in the number of therapeutic phlebotomies required per patient by those receiving ropegrIFNα-2b compared with those receiving phlebotomy alone (2.8 vs 3.8; *P*=.029), with an increase in ferritin concentrations. Additionally, there was a significant decrease in leukocytosis and thrombocytosis without a significant impact on baseline spleen size. There were significantly more AEs in those treated with ropegrIFNα-2b (78%) than in those treated with standard therapy alone (42%). Key AEs included low-grade neutropenia (10%) and flu-like symptoms (16%) in those treated with ropegrIFNα-2b. The rate of grade 3 AEs was low in both study arms. Because the patients enrolled were all at low risk for thrombotic events and the median follow-up time was 12 months, it is difficult to predict the longer-term clinical significance of treatment with ropegrIFNα-2b in low-risk individuals based on this trial. Accordingly, the utility of ropegrIFNα-2b and HU in low-risk patients remains debated; however, this trial does demonstrate safety and tolerability, as well as a signal of efficacy in terms of a reduction in phlebotomy requirement, normalization of leukocyte and platelet counts, and normalization of ferritin levels. Longer-term follow-up will be needed to confirm an impact on thrombotic events or disease progression.

Ruxolitinib

Ruxolitinib is effective in reducing splenomegaly in patients with PV and is approved for patients with inadequate response to or who are intolerant of HU.[82] The randomized phase 3 RESPONSE study enrolled patients who were phlebotomy-dependent with splenomegaly. Patients were randomly assigned to receive either ruxolitinib at 10 mg twice daily (n=110) or standard therapy, which was overwhelmingly HU (n=112). The primary outcome was HCT control (absence of therapeutic phlebotomy) and a reduction of at least 35% in spleen volume (SVR_{35}) at week 32. This composite outcome was achieved in 20.9% of patients treated with ruxolitinib vs 0.9% of those treated with standard therapy. An SVR_{35} was achieved in 38% of patients in the experimental arm vs 1% of those in the standard-therapy arm.[83] It is important to note that because HU increases mean corpuscular volume, which in turn increases HCT, the use of HU may lead to unnecessary phlebotomy through influence on red cell size rather than red cell count. This would therefore skew improvement in HCT control toward the experimental arm in this trial. The RBC count was not systematically followed in this trial.

Similarly, the MAJIC-PV study evaluated patients with PV with refractory disease or intolerance to HU. This randomized phase 2 study included individuals with or without splenomegaly. Patients were randomized to receive either ruxolitinib or best available therapy (BAT). The primary outcome was CR within 1 year. Ruxolitinib demonstrated an improvement in CR compared with BAT (43% vs 26%; *P*=.02). Event-free survival, which was one of the secondary outcomes and included major thrombosis, hemorrhage, disease progression, or death, was also shown to be improved in the treatment arm (HR, 0.58; *P*=.03).[18] Together, these studies suggest that ruxolitinib impacts spleen and symptom burden and potentially the risk of both thrombotic events and disease progression.[18] The MAJIC-PV is the first trial to demonstrate the ability of ruxolitinib to attain molecular responses that were correlated with clinical outcome measures. At a median follow-up of 36 months, a greater than 50% reduction in *JAK2* V617F VAF occurred in 56% (39/70) vs 25% (14/57) of patients treated with ruxolitinib and BAT, respectively. In 3 selected patients who had a greater than 90% reduction in *JAK2* V617F VAF, there was a significant reduction in the percentage of hematopoietic stem and progenitor cells (HSPC) that were *JAK2* V617F–positive by single-cell genotyping from baseline to follow-up. With these data, we may reconsider the patient population in which we implement ruxolitinib.[18] Rather than utilizing it in a HU-refractory population with high symptom burden and splenomegaly, it may be worth investigating in earlier treatment lines with high-

risk disease, and even in those with lower-risk disease. The ongoing MITHRIDATE study in the United Kingdom plans to address this question in a phase 3 randomized, open-label trial comparing ruxolitinib with either hydroxycarbamide or IFNα in high-risk PV patients in the frontline setting (NCT04116502). Of course, it is important to note the toxicities associated with ruxolitinib across the clinical studies that must be considered. Notable toxicities include myelosuppression and infection risk, particularly with herpes zoster. Ruxolitinib has also been associated in some studies with an increased risk of developing second primary malignancies, particularly lymphoma and nonmelanoma skin cancers.[84] In practice, the use of ruxolitinib remains limited to patients with refractory symptoms and lack of splenomegaly control despite HU or pegrIFNα-2a or 2b.

Moving forward, it is also important to consider the potential synergies of approved therapies. A phase 2 study evaluating the combination of ruxolitinib (5-20 mg twice daily) and low-dose pegrIFNα-2a (35-45 μg) demonstrated tolerability and efficacy in a small cohort of patients with PV. This trial enrolled patients with both PV and MF. Thirty-two patients with intolerance to treatment or refractory PV despite treatment with pegrIFNα-2a were enrolled. Ten of 32 patients (32%) achieved a response by 2013 IWG-MRT criteria. The combination was tolerable.[85] Although these data from the small cohort of PV patients are encouraging, the combination of pegrIFNα-2a or -2b plus ruxolitinib requires a larger prospective evaluation before it can be considered a standard of care approach. For now, ruxolitinib and pegrIFNα-2a continue to be used as monotherapy.

Novel Therapeutics for Hematocrit Control

In addition to requiring lengthy and often inconvenient clinic visits, therapeutic phlebotomy can lead to symptomatic iron deficiency. Iron deficiency related to therapeutic phlebotomy often leads to exacerbation of baseline disease–related headaches, fatigue, and concentration deficits.[86] Accordingly, alternate mechanisms of reducing HCT are in development. The most advanced is the hepcidin mimetic rusfertide (PTG-300). Like hepcidin, PTG-300 binds to ferroportin and downregulates iron absorption and mobilization, thereby downregulating erythropoiesis and effectively reducing HCT and the need for therapeutic phlebotomy while leading to a normalization of serum ferritin, suggesting a normalization and redistribution of systemic iron stores.[87-90] Phase 2 trials evaluated ELN low-risk and high-risk PV patients with persistent phlebotomy requirements and demonstrated the ability to control HCT without the need for phlebotomy. Treatment with weekly self-administered subcutaneous PTG-300 nearly eliminated the need for therapeutic phlebotomy in all patients, and serum ferritin levels trended toward normal.[88] Preliminary data from the ongoing phase 2 trial have also demonstrated the ability to control HCT without the need for phlebotomy in PV patients with poorly controlled baseline HCT.[89,90] Owing to a brief clinical hold imposed by the FDA over concerns regarding the increased risk of skin cancer in animal models, treatment was interrupted for patients enrolled in the clinical trial. During interruption, both hematologic parameters and symptoms acutely worsened. Patients who achieved phlebotomy independence became phlebotomy-dependent during interruption. Additionally, patients noted a worsening of fatigue that rapidly resolved after resuming subcutaneous therapy.[91] The treatment was well-tolerated. The most common treatment-emergent AEs were injection site reactions and fatigue. There were no grade 4 or 5 treatment-emergent AEs.

In part 2 of the phase 2 REVIVE study, patients receiving PTG-300 were randomly assigned to either continue the study agent or receive a placebo for 12 weeks. Fifty-three patients were randomized and completed part 2. Patients in the rusfertide arm demonstrated a significant improvement in maintenance of response and absence of need for therapeutic phlebotomy compared with those randomized to placebo ($P<.0001$).[92] Together, these data suggest a possible targeted therapeutic candidate that effectively controls HCT while maintaining normal iron stores.

The phase 3 VERIFY trial (NCT05210790) is an ongoing randomized, placebo-controlled trial evaluating PTG-300 in PV. Importantly, unlike nonspecific cytoreductive and myelosuppressive agents, PTG-300 downregulates erythropoiesis and does not affect WBC and platelet production. It is important to note that this compound does not affect the underlying malignant clone, and therefore may leave patients vulnerable to potential thrombotic complications of the disease. It also may not mitigate the risk of disease progression.

Conclusion

As our knowledge of disease biology and treatment options grows and evolves, it will be essential to revisit our risk stratification protocols along with our treatment paradigms and response criteria. The 2013 IWG-MRT response criteria include clinical criteria that dictate the normalization of cytologic abnormalities but lack prospective validation. The thrombotic consequences of poorly controlled HCT have been well studied and demonstrated in large prospective clinical trials. There are limited data to support that normalization of thrombocytosis results in improved clinical outcomes, and although leukocytosis appears to have adverse prognostic significance, the clinical benefits of normalizing

leukocytosis remain unknown but worthy of investigation. There is a growing body of evidence that treatments that target and reduce the underlying malignant clone burden lead to decreased risk of thrombotic complications and disease transformation. Response criteria and clinical guidelines should be updated to incorporate only high-quality evidence-based measures. Even with significant challenges in obtaining prospective data in PV, including the relative rarity of this disease and the prolonged time from diagnosis to thrombotic events or disease transformation, it is vital to invest the resources to answer these questions to determine how best to evaluate and manage our patients with PV.

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