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MYELOPROLIFERATIVE DISORDERS (C HARRISON, SECTION EDITOR)

How We Identify and Manage Patients with Inadequately Controlled Polycythemia Vera

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Abstract Polycythemia vera (PV) is a chronic myeloproliferative neoplasm (MPN) characterized by an overactive Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway through mutations in *JAK2* exons 12 or 14 (*JAK2* V617F). The dominant clinical characteristics include erythrocytosis (with or without leukocytosis/thrombocytosis), thrombotic events, and symptoms. Increased risk of mortality is mainly caused by thrombotic events and progression to post-polycythemia vera myelofibrosis (PPV-MF) or secondary acute myeloid leukemia (sAML). The most important prognostic factors include age and a history of thrombotic events, although recent evidence has indicated that leukocytosis and additional cytogenetic aberrations may also be of significant prognostic value. First-line therapies include aspirin and phlebotomies, which significantly reduce the incidence of thrombotic events and prolong survival. Cytoreductive treatment with hydroxyurea (approved) and conventional or pegylated interferon-α (effective, but not approved in many countries) is initiated for high-risk or inadequately controlled disease, e.g., uncontrolled hematocrit, leukocytosis, thrombocytosis, thrombotic events, splenomegaly, or symptoms. However, some patients may not receive initial benefit from first-line therapy or may become resistant or intolerant in due course. Although second-line treatment options are limited, clinical trials have shown the efficacy of ruxolitinib toward improving blood counts, enlarged spleen, and symptoms and potentially reducing thrombotic events. Identification of patients with uncontrolled PV is important for clinical care, as such patients have a high risk of complications, and future studies with JAK inhibitors or other agents alone or in combination are needed to test their potential to reduce rates of thrombotic events and transformation to PPV-MF or sAML.

Keywords Polycythemia vera · Hydroxyurea · Interferon · Ruxolitinib

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Introduction

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm (MPN) primarily characterized by erythrocytosis [1–5], and often leukocytosis and/or thrombocytosis [2, 3, 5]. Elevated blood counts result from a trilineage proliferation of hematopoiesis in the bone marrow (BM), with most patients (≈98–99 %) having mutations in the Janus kinase 2 (*JAK2*) gene [6–8]. The point mutation *JAK2* V617F in exon 14 appears to be the driver mutation for PV [9–14] and is present in ≈96 % of patients [6, 7], while mutations (small deletions or insertions) in exon 12 of *JAK2* are found in approximately 3 % of patients with PV [6–8].

Polycythemia vera is diagnosed slightly more often in men than women, with the median age at diagnosis being 60 years [15]; however, ≈10 % of patients are <40 years at diagnosis [16•]. Thrombotic events are a major complication of the disease, accounting for 45 % of all deaths [4] and may be found at atypical sites (e.g., splanchnic and cerebral veins) [17, 18]. The course of PV (Fig. 1) begins with an erythrocytotic phase [19] during which patients may be asymptomatic or

experience symptoms (e.g., fatigue, headache, paresthesia, arthralgia; Fig. 2) [18]. Over time, the disease commonly evolves to an advanced phase, with patients exhibiting disease features that may include fatigue, severe pruritus, bone pain, night sweats, and in some cases, splenomegaly [16•, 18, 22]. Additionally, patients with a long disease duration (usually >10 years) may progress to post-polycythemia vera myelofibrosis (PPV-MF; HR, 15.24; 95 % CI 4.22–55.06 [23]; 10- and 15-year cumulative risk, 2.3 and 6 %, respectively [16•]), with a median time to progression of 13 years [24]. In this phase, patients have increased BM fibrosis and worsening splenomegaly, with some patients exhibiting progressive cytopenias [25]. Furthermore, patients are at risk of evolution to secondary acute myeloid leukemia (sAML) with a 15-year cumulative risk of 7 % [4, 15, 23, 24].

For many patients, the disease is easily managed. However, some patients have a suboptimal response to available therapies, which can manifest as uncontrolled hematocrit (HCT), thrombotic events, leukocytosis, thrombocytosis, symptoms, or increasing spleen size and lead to what we have termed "inadequately controlled PV." This can occur at any phase of the disease and can ultimately result in reduced survival. In this review, we will describe how we identify these patients and discuss potential therapeutic strategies to improve management of their PV.

Current Risk Stratification and Initial Treatment Strategies

Traditionally, risk classification in PV is determined by age and history of thrombotic events [26, 27]. Patients <60 years with no history of thrombotic events are considered low risk, whereas patients aged ≥60 years and/or who have prior occurrences of thrombotic events are considered high risk. An intermediate-risk category that includes patients <60 years of age with no history of thrombotic events but with cardiovascular risk factors (e.g., hypertension, hypercholesterolemia, diabetes, obesity, smoking) has been proposed but not formally defined [28, 29]. Higher risk

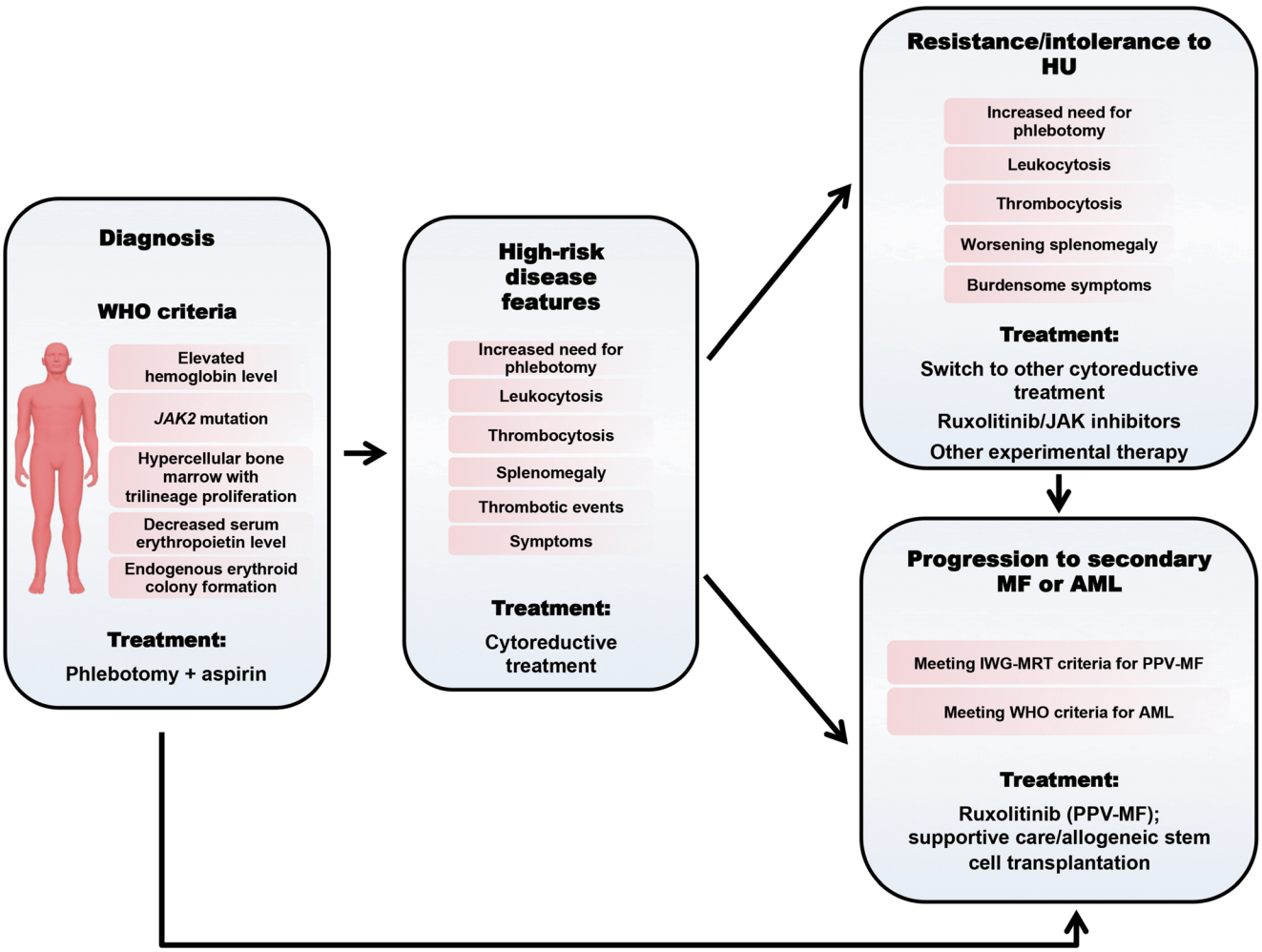

Fig. 1 Progression of polycythemia vera. *AML* acute myeloid leukemia, *HU* hydroxyurea, *IWG-MRT* International Working Group-Myeloproliferative Neoplasms Research and Treatment, *JAK2* Janus kinase 2, *MF* myelofibrosis, *PPV-MF* post-polycythemia vera myelofibrosis, *WHO* World Health Organization

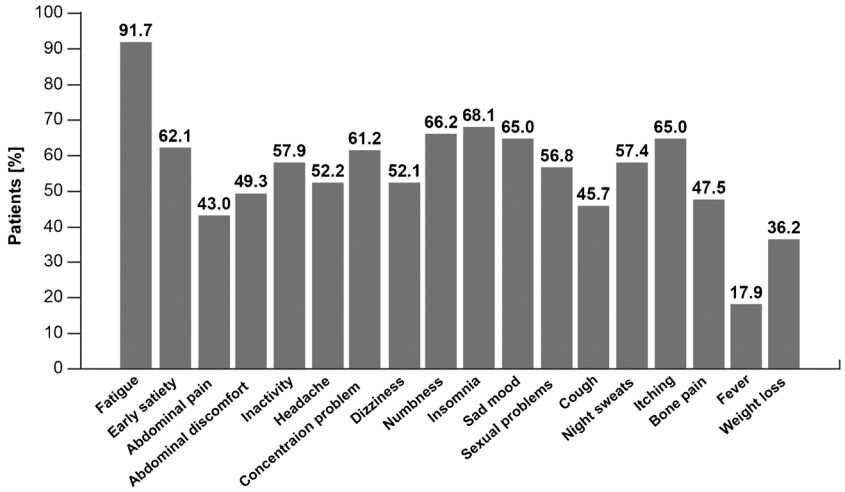

Fig. 2 Prevalence of symptoms in polycythemia vera (PV) [20, 21]

patients have reduced survival compared with low-risk patients [16•]. An analysis of overall survival (OS) in a large cohort of treated patients with PV ($N = 1545$) found median survival to be 28, 19, and 11 years for low-, intermediate-, and high-risk patients, respectively, based on a prognostic model that used age, leukocytosis, and venous thrombosis as variables (Fig. 3) [16•]. However, the impact of arterial thrombosis on survival was not assessed in this model and will require further clarification.

Given that thrombotic events are such a major complication of the disease, reducing thrombotic risk is a main goal of therapy. Treatment strategies target HCT <45 %, a treatment goal that was recently supported by the CYTO-PV (Cytoreductive Therapy in Polycythemia Vera) study ($N = 365$), in which aggressively targeting HCT <45 % was shown to reduce the risk of major thrombotic events and death from cardiovascular causes [30••]. In this study, patients randomized to the high-HCT group (target HCT 45–50 %) had a fourfold increase in the rate of death from cardiovascular causes or major thrombosis compared with those in the low-HCT group (target HCT <45 %), as well as a higher incidence of thrombosis. Treatment recommendations are based on the patient's risk stratification. All patients are usually treated for optimization of cardiovascular risk factors with low-dose aspirin and phlebotomy [31], while hydroxyurea (HU) and interferon-α (IFN-α) [25, 27] are the recommended first-line treatments for high-risk patients [25, 27]. Currently, there are no recommendations for PV-specific therapies for intermediate-risk patients, making adequate treatment of this patient subgroup difficult.

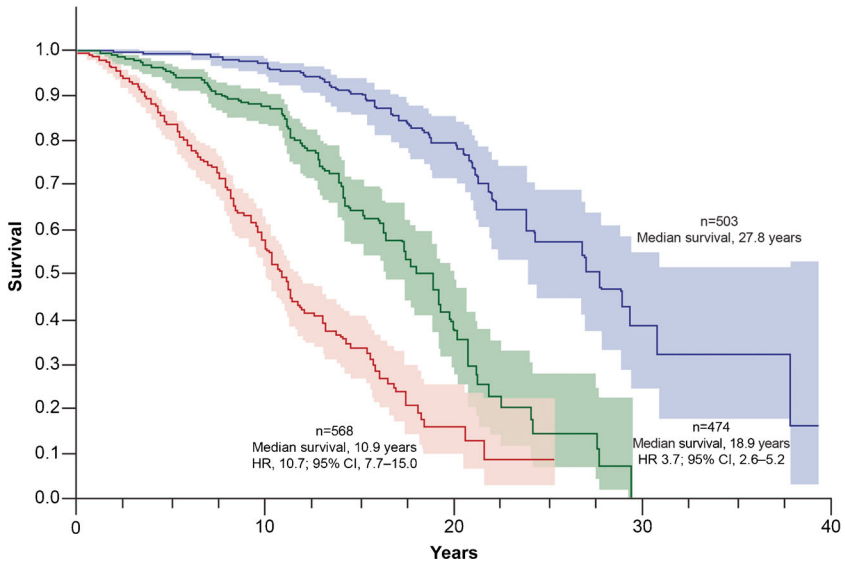

Fig. 3 Risk-stratified survival in patients with PV ($N = 1545$) [16•]. Adverse points were assigned to age ≥67 years (5 points), age 57–66 years (2 points), white blood cell count $\geq 15 \times 10^9$/L (1 point), and venous thrombosis (1 point): low-risk (0 points), intermediate-risk (1 or 2 points), and high-risk (≥3 points). Using mature survival data from a subgroup of patients seen at the Mayo Clinic ($n = 337$), median survival was calculated to be 26, 15, and 8.3 years for low-, intermediate-, and high-risk patients, respectively [16•]; the model was subsequently validated in the entire study cohort ($N = 1545$). Reprinted by permission from Macmillan Publishers Ltd: Tefferi et al. [16•]

Identifying Inadequately Controlled PV

For a large proportion of patients, following the recommendations just described results in well-managed PV, with controlled HCT, hematologic parameters, spleen, and symptoms. Other patients, however, develop resistance or intolerance to treatment (Table 1). These patients have suboptimal responses which can manifest in different ways, including elevated HCT, the occurrence of thrombotic events, persistent leukocytosis/thrombocytosis, increasing spleen size, and burdensome symptoms. These indicators of inadequately controlled disease are not confined to any one phase of treatment and may occur at any time during the course of the disease. These patients are at an increased risk of thrombosis [32–35], reduced quality of life (QOL) [22, 36–38], and shortened survival [16•, 33, 39, 40•, 41], and thus are usually considered high-risk. Patients with intolerance require a change of therapy if the intolerance is sufficiently severe; however, intolerance itself has not been shown to result in the same increased risk of thrombosis or shortened survival.

Given the results of the CYTO-PV study [30••], the two most important indicators of inadequately controlled PV are the presence of uncontrolled HCT and the occurrence of thrombotic events. As discussed above, tight control of HCT is imperative for reducing the risk of thrombotic events and, therefore, the risk of death. Other clinical features, such as persistent leukocytosis (leukocyte count $> 10 \times 10^9$/L) and/or thrombocytosis (platelet count $> 400 \times 10^9$/L), are also indicative of inadequately controlled disease [27]. Leukocytosis at PV diagnosis has been found to be prognostic for thrombosis [34, 35], leukemic transformation [16•, 42, 43], and survival [16•, 33, 39, 41, 44]. Similarly, studies have found a correlation between persistence of leukocytosis and adverse outcomes in PV, e.g., thrombosis [30••], including arterial [32, 35] and venous thrombosis [33], and hematologic transformation and shorter survival [40•], regardless of a patient's risk category. In the CYTO-PV study, patients in the high-HCT group had significantly higher leukocyte counts than patients in the low-HCT group, suggesting that leukocytosis despite treatment (which in this study was a mixture of phlebotomy only or cytoreductive therapies such as HU) could have contributed to the higher rates of thrombosis seen in the high-HCT group [30••]. A subsequent multivariable, time-dependent analysis by Barbui and colleagues [45] found that the risk of thrombosis increased significantly in patients with a leukocyte count $> 11 \times 10^9$/L. Interestingly, an increase in the risk of thrombosis was also seen in patients with a leukocyte count $> 7 \times 10^9$/L; however, this increase was not statistically significant. These results, while interesting, need to be interpreted bearing in mind that patients differed not only in leukocyte count but also in HCT values. Similarly, findings from the ECLAP study showed that leukocytosis was significantly associated with vascular risk in patients, regardless of treatment [32]. Furthermore, leukocytosis despite treatment with HU was associated with a higher risk of hematologic transformation ($P = .004$) and reduced survival ($P = .007$) in a study evaluating the utility of the European LeukemiaNet (ELN) response criteria in patients treated with HU (median follow-up, 7.2 years) [40•]. This last study also showed that, although there is no clear association between elevated platelet counts and major thrombotic events, failing to achieve an ELN-defined platelet response with treatment is associated with a higher risk of thrombosis and bleeding in patients with PV [40•]. These findings suggest that successful management of PV should include a response in leukocyte and platelet counts as well as achieving HCT < 45 %.

Another sign of inadequately controlled disease is that of burdensome and intractable symptoms, but these have not been

Table 1 Identifying resistance/intolerance to therapies

Drug	Clinical feature indicative of resistance/intolerance
All therapies (after 3 months of treatment)	Need for phlebotomy to achieve HCT < 45 %[a]
	Leukocytosis (leukocyte count $> 10 \times 10^9$/L)
	Thrombocytosis (platelet count $> 400 \times 10^9$/L)
	Failure to achieve a >50 % reduction in palpable splenomegaly measuring >10 cm from the left costal margin
	Neutropenia (absolute neutrophil count $< 1.0 \times 10^9$/L; platelet count $< 100 \times 10^9$/L; hemoglobin < 10 g/dL) at the lowest dose required to achieve a complete or partial clinicohematologic response
	Unbearable disease-related symptoms (e.g., pruritus)
	Thrombosis or hemorrhage on therapy
HU	Leg ulcers, mucocutaneous manifestations, fever, pneumonitis, or other HU-related nonhematologic toxicities at any dose of HU
IFN-α	Depression, flu-like symptoms, neuropsychiatric symptoms, or autoimmune problems
Ruxolitinib	Anemia, thrombocytopenia, neutropenia, risk of infections, and risk of nonmelanoma skin cancer

[a] In the authors' view, this is not a strong indicator of resistance or intolerance, but clinicians should be mindful of frequency of venesections. *HCT* hematocrit, *HU* hydroxyurea, *IFN-α* interferon-α

linked to reduced survival or increased risk of thrombosis. Several studies have shown that patients with PV have a considerable symptom burden (Fig. 2) [20, 21, 46]. Fatigue is the most frequently reported symptom, based on studies using the Myeloproliferative Neoplasm Self-Assessment Form (MPN-SAF) [20–22], a questionnaire designed to capture patient-reported symptoms in patients with MPN [21] that has been used in several trials to measure symptomatic response to treatment. Pruritus, another common symptom, is characterized by strong, and in some cases, unbearable itching, stinging, tingling, or burning sensations, usually following contact with water, and is considered by many to be the most troublesome symptom [21, 38, 47]. Additionally, approximately 30 to 40 % of patients present with splenomegaly and splenomegaly associated symptoms during the course of the disease [16•].

Conventional Treatment Options for Patients with Inadequately Controlled PV

First-Line Treatment Options

Patients who are inadequately controlled do not always have many treatment options. Patients who show elevated HCT can increase the frequency of phlebotomies. However, frequent phlebotomies are not always well tolerated and may lead to iron deficiency, which has been associated with other complications such as restless legs syndrome [48] and cognitive dysfunction [49]. Alternatively, patients may also begin treatment with HU or IFN-α. In some instances, however, patients already being treated with HU and/or IFN-α may become resistant to or intolerant of these therapies. Approximately one fourth of patients with PV develop ELN-defined resistance (11 %) or intolerance (13 %) to HU (Table 2) [40•, 50], and are consequently at a high-risk of thrombosis and bleeding (34.7 and 16.8 per 1000 person-years, respectively) compared with those responding to HU per ELN criteria (28.3 vs 12.8 per 1000 person-years, respectively) [40•]. Furthermore, HU-resistance is associated with an increased risk of death and transformation to PPV-MF or sAML ($P < .001$) [40•]. There are currently no ELN criteria for IFN-resistance or -intolerance.

Patients resistant to or intolerant of HU or IFN-α require increasing treatment doses and/or more frequent phlebotomies (>1/month) to achieve HCT control. Other patients achieve HCT control but have uncontrolled leukocytosis or thrombocytosis and/or symptoms, or experience toxicity (e.g., mucositis, leg ulcers, and skin cancer with HU, or flu-like symptoms, neuropsychiatric symptoms, and autoimmune problems with IFN [51]). However, it is sometimes difficult to discern whether these toxicities are a consequence of disease progression and/or cytoreductive treatment.

Second-Line Treatment Options

IFN-α is the ELN-recommended second-line therapy in patients who have become resistant to or intolerant of HU [27] in part because it is not considered leukemogenic [43], an important characteristic considering that some drugs administered after HU may increase the risk of patients developing sAML [43], although the leukemogenic effect of HU itself remains unproven. However, IFN-α has never been formally assessed in a randomized phase 3 study as second-line therapy in HU-resistant or HU-intolerant patients. Studies have shown that

Table 2 ELN definition of resistance/intolerance to HU in patients with polycythemia vera

Dose	Clinical feature
At ≥2 g/day of HU (after 3 months of treatment):	• Need for phlebotomy to keep HCT <45 %, *or* • Uncontrolled myeloproliferation: (i.e., platelet count >400 × 10^9/L *and* leukocyte count >10 × 10^9/L), *or* • Failure to reduce massive[a] splenomegaly by more than 50 % as measured by palpation, *or* • Failure to completely relieve symptoms related to splenomegaly, *or*
At the lowest dose of HU required to achieve a complete or partial clinico-hematologic response[b]:	• Absolute neutrophil count <1.0 × 10^9/L, *or* • Platelet count <100 × 10^9/L, *or* • Hemoglobin <100 g/L, *or*
At any dose of HU:	• Presence of leg ulcers or other unacceptable HU-related non-hematologic toxicities, such as mucocutaneous manifestations, skin cancer, gastrointestinal symptoms, pneumonitis, or fever

Resistance/intolerance to HU requires meeting at least one of the listed clinical features at the indicated dose. From [50]

HCT hematocrit, *HU* hydroxyurea

[a] Spleen extending >10 cm from the costal margin

[b] Complete response was defined as HCT <45 % without phlebotomy, platelet count ≤400 × 10^9 /L, leukocyte count ≤10 × 10^9 /L, and no disease-related symptoms. Partial response was defined as HCT <45 % without phlebotomy, or response in ≥3 other criteria

IFN-α is very effective in reducing rates of thrombosis [52, 53], controlling symptoms [54, 55], and eliciting complete hematologic response [55, 56] or substantial decreases in *JAK2* V617F allele burden [56]. However, IFN-α is associated with significant treatment-related toxicities (e.g., flu-like symptoms, depression, autoimmune disorders) [18] leading to permanent discontinuation in 20 to 40 % of patients [18, 57] and preventing its use in elderly patients and in those with some preexisting psychological and immune disorders [4, 58].

A newer pegylated formulation (PEG-IFN-α; Pegasys) has been reported to be more tolerable, to result in high rates of hematologic responses, and to reduce the *JAK2* V617F allele burden in phase 2 trials of patients with PV who are either treatment naive or have been previously treated with phlebotomies or cytoreductive treatment [58, 59]. In one study, all evaluable patients who were treated first line (37/40) had a hematologic response, with 95 % achieving complete clinicohematologic response; complete molecular response was achieved in seven patients [58]. Overall, 24 % of patients discontinued treatment due to toxicity. In a second study in the second-line setting (PV, $n=40$; essential thrombocythemia [ET], $n=39$), the complete hematologic response was 76 % and the complete molecular response was 18 % in patients treated with PEG-IFN-α-2a [59]; 20 % of patients discontinued due to treatment-related adverse events. PEG-IFN-α is currently being tested in two phase 3 trials for the treatment of PV and, in the future, may become a viable option for some patients [60]. A randomized, open-label trial through the Myeloproliferative Disorders Research Consortium is evaluating the safety, toxicity, and tolerability of PEG-IFN-α–2a vs HU in high-risk patients with PV or ET (ClinicalTrials.gov, NCT01259856). The primary outcome will be hematologic response rates in the two study arms. PROUD-PV (Pegylated Interferon Alpha-2b Versus Hydroxyurea in Polycythemia Vera; ClinicalTrials.gov, NCT01949805), the second phase 3 study, is comparing the efficacy and safety of the novel monopegylated IFN-α-2b against HU in high-risk *JAK2* V617F-positive PV. The primary outcome is peripheral blood count remission and normal spleen size after 1 year of treatment. However, neither of these studies is evaluating the use of IFN-α in patients who are HU-resistant or HU-intolerant.

Anagrelide is another recommended second-line therapy for the treatment of PV and is also considered nonleukemogenic. Anagrelide, however, only has platelet-reducing activity [27], and its combination with HU may be necessary in patients with progressive disease or those with uncontrolled HCT, leukocytes, symptoms, or platelet counts. The longest follow-up study of HU plus anagrelide found that a low-dose combination in patients with PV or ET whose disease was resistant or refractory to single-agent HU or anagrelide led to complete remission in 8 of 12 and partial responses in 3 of 12 patients, with a median platelet count reduction of 45 % [61]. Additionally, the low-dose combination therapy was associated with few adverse events. However, anagrelide is not approved in the EU as combination therapy and is only approved as treatment for MPN in patients with ET. Additionally, in patients with ET, anagrelide treatment has been associated with cardiac toxicity, increased bleeding when combined with aspirin, and an increased risk of transformation to secondary MF [62].

Busulfan has also been recommended as a second-line therapy in patients who are HU refractory [25] because it may lead to durable hematologic responses and was shown to reduce the *JAK2* V617F allele burden, albeit in a small patient cohort [63]. Busulfan was recently evaluated retrospectively as second-line therapy in patients with PV or ET showing signs of resistance to HU ($n=36$) [64]. Complete clinicohematologic response was achieved in 83 % of patients with an 87 % probability of sustained CR at 1 year and 62 % at 2 years. Fifty percent of patients discontinued because they achieved clinicohematologic response; others discontinued due to hematologic toxicity ($n=8$) and transformation to sAML ($n=1$). The rate of partial molecular response in evaluable patients with PV was 60 % ($n=3/5$); no patient achieved a complete molecular response. At 2 years, the probability of survival was 85 % and the probability of thrombosis 11 %. However, although transformation to sAML was only seen in 2 of these 36 patients, there is significant concern regarding leukemogenicity when busulfan is given following treatment with HU [43]; thus, busulfan continues to be recommended only in patients >70 years of age [27].

Pipobroman or phosphorus 32 (^{32}P) might also be considered [27], although both agents are not widely available in all countries and only a few patients are treated with these agents. A recent, large multicenter study analyzing survival and leukemic transformation in 1545 patients with PV reported that approximately 11 % of assessed patients had been treated with pipobroman as a single agent or in combination with other therapies; 4.2 % had been treated with alkylating drugs, including ^{32}P [16•]. Like busulfan, these agents may be leukemogenic [39, 40•, 43, 64], and in the case of pipobroman, patients treated with this agent have a cumulative incidence of treatment-related myelodysplastic syndrome/sAML of 13, 34, and 52 % at 10, 15, and 20 years ($P=.004$), respectively [65]. Therefore, these therapies are usually reserved for older patients or those with a short life expectancy [39, 40•, 43, 64].

Patients with persistent leukocytosis may be difficult to treat, especially those who are also HU-resistant or HU-intolerant. Although a response definition for patients with leukocytosis and/or thrombocytosis has been established by the ELN [66], there are no clear recommendations for which therapy to use when leukocytosis or thrombocytosis persists despite treatment. Options include IFN-α, busulfan, and pipobroman, as discussed earlier. Most patients should continue taking low-dose aspirin as antiplatelet therapy, based on the findings from the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) study [31]. However, aspirin should probably be withdrawn if a patient's platelet count exceeds 1500×10^9/L [27] because patients with extreme thrombocytosis may be at risk of

developing an acquired von Willebrand defect and in turn, aspirin-associated bleeding [25]. In patients with extremely elevated platelet counts, treatment with cytoreductive agents (e.g., anagrelide plus HU) may be required before aspirin is instituted, but it is important to note that the concomitant use of anagrelide and aspirin may be associated with increased bleeding events [67]. For those patients with severe PV-related symptoms, there is little and conflicting evidence regarding efficacy of current treatment options and their ability to impact symptom burden [20, 21, 36, 38, 56, 68]. However, very few of these studies prospectively tested the efficacy of standard therapies in treating PV-associated symptoms or evaluated disease burden in the same patients before and after treatment, making it difficult to accurately determine treatment-related changes in symptom burden.

Ruxolitinib

Although not currently a part of the ELN guidelines, the JAK1/JAK2 inhibitor ruxolitinib was recently approved for the treatment of patients with PV who are resistant to or intolerant of HU. This approval was based on the findings of the phase 2 Study 256 [69] and the phase 3 RESPONSE (Randomized Study of Efficacy and Safety in Polycythemia Vera with JAK Inhibitor INCB018424 versus Best Supportive Care) study [70••]. Results from Study 256 ($n=34$; ClinicalTrials.gov, NCT00726232) showed that ruxolitinib led to responses in blood counts, including HCT, meaningful reductions in spleen size, and improvements in symptoms in patients with PV who were HU-resistant or HU-intolerant [69]. Most patients (97 %) achieved HCT <45 % without phlebotomy and normalization of leukocytosis (73 and 76 % of patients with baseline leukocyte count $>15\times10^9$ and $>10\times10^9$/L, respectively) [69]. Additionally, 74 % of patients with elevated platelets achieved a sustained platelet response ($<400\times10^9$/L). Thrombocytopenia and anemia were the most common adverse events, with grade 3/4 events occurring in 9 % of patients each; both adverse events were managed with dose modifications.

Based on these results, the phase 3 RESPONSE study ($N=222$; Clinicaltrials.gov, NCT01243944) was initiated [70••]. RESPONSE assessed the efficacy and safety of ruxolitinib vs standard therapy in patients with PV who had an inadequate response to or had unacceptable side effects from HU (i.e., HU-resistant or HU-intolerant) and who had splenomegaly. The primary endpoint, a composite of the percentage of patients who achieved both HCT control without phlebotomy between weeks 8 and 32 and a ≥35 % reduction in spleen volume from baseline at week 32, was achieved in 21 % of patients in the ruxolitinib group vs 1 % of those receiving standard therapy ($P<.001$). Higher proportions of patients in the ruxolitinib arm achieved HCT control (60 vs 20 %). Additionally, patients in the ruxolitinib arm required fewer phlebotomies to maintain HCT control compared with those who received standard therapy (i.e., the "best available therapy" arm of the trial). A total of 19.8 and 62.4 % of patients receiving ruxolitinib or standard therapy, respectively, underwent ≥1 phlebotomy; 2.8 and 20.2 %, respectively, underwent ≥3 phlebotomies. Furthermore, patients receiving ruxolitinib had fewer occurrences of thrombotic events (1 [portal vein thrombosis] vs 6 [myocardial infarction, deep vein thrombosis, pulmonary embolism, splenic infarction, thrombophlebitis, and thrombosis], respectively). However, neither the number of phlebotomies nor the rate of thrombotic events were predefined primary endpoints, limiting their interpretation.

Ruxolitinib was well tolerated, and the most common adverse events were anemia (grade 3/4, 2 %) and thrombocytopenia (grade 3/4, 5 %), consistent with findings from Study 256; the corresponding rates in the standard therapy arm were 0 and 4 %. Herpes zoster infection (all cases were grade 1 or 2) was reported in 6.4 % of patients randomized to ruxolitinib and 0 % of patients in the standard therapy arm. Overall, the rate of infections was 41.8 % in the ruxolitinib group and 36.9 % in the standard therapy arm. Nonmelanoma skin cancer was diagnosed in four patients in the ruxolitinib group and in two patients in the standard therapy arm; the majority of patients (all but 1) had a history of nonmelanoma skin cancer or precancerous skin lesions. Three patients randomized to ruxolitinib developed PPV-MF and 1 patient received a diagnosis of sAML. PPV-MF developed in one patient assigned to standard therapy; additionally, two patients received a diagnosis of PPV-MF after crossover, one of whom had progression to sAML. This is perhaps disappointing, as these are events that ideally would have been prevented or reduced. Overall, ruxolitinib proved superior to standard therapy in inducing HCT control, reducing spleen size, and improving symptoms and QOL in this patient population.

Despite these intriguing results, there were some limitations in the design of the study. Eligible patients were required to have splenomegaly, a disease characteristic that is infrequent and is usually associated with advanced disease. Additionally, 59 % of patients in the standard therapy arm were treated with HU although most patients (54 %) were considered HU-intolerant. Furthermore, clinicohematologic response according to ELN criteria (HCT < 45 % without phlebotomy, response in platelet and leukocyte count, normal spleen size, and no disease-related symptoms) [71] was not reported, although more patients treated with ruxolitinib than those treated with standard therapy achieved a response in HCT, platelet count, and leukocyte count (24 vs 9 %). Moreover, BM biopsies were not requested at study entry and were used only to confirm progression to PPV-MF, preventing the reporting of normalization of BM features as well as the identification of those who may have developed PPV-MF (although PPV-MF that required phlebotomy) at or shortly after study enrollment. In addition, the impact of study endpoints, in particular reduction of splenomegaly and freedom from phlebotomy, could be questioned in terms of their disease modification.

Additional trials evaluating ruxolitinib in this patient population include the RESPONSE2 study (Clinicaltrials.gov, NCT02038036) and the MAJIC (Randomised Study of Best

Available Therapy Versus JAK Inhibition in Patients With High Risk Polycythaemia Vera or Essential Thrombocythaemia Who Are Resistant or Intolerant to Hydroxycarbamide) study (EudraCT, 2011-005279-18). RESPONSE2 is a phase 3 trial that will study the efficacy and safety of ruxolitinib in patients without splenomegaly who are HU-resistant or HU-intolerant. The MAJIC study is a UK-specific, randomized, phase 2 study that will assess the efficacy and safety of ruxolitinib vs best available therapy in patients with high-risk PV (or ET) who are HU-resistant or HU-intolerant and who have an enlarged spleen. Alternatively, patients may be candidates for investigational studies with JAK inhibitors other than ruxolitinib, as well as deacetylase (HDAC) inhibitors.

The RESPONSE study suggests that ruxolitinib may also be an effective treatment for persistent leukocytosis and thrombocytosis. At week 32, more patients randomized to ruxolitinib had a leukocyte count $\leq 15 \times 10^9$/L than patients randomized to standard therapy (70 vs 43 %, respectively) [72]. Similarly, more patients receiving ruxolitinib had a platelet count $\leq 600 \times 10^9$/L (82 vs 64 %) [72]. Complete hematologic remission (CHR; normalization in HCT, leukocytes, and platelets) was achieved in 24 % of ruxolitinib-treated patients; in contrast, only 9 % of patients treated with standard therapy achieved this result ($P = .003$). Given these data, JAK inhibitors could also play an important role in the treatment strategy for patients with persistent leukocytosis and/or thrombocytosis. However, the role of leukocytosis in PV remains unclear, and it is still unknown whether therapies that control leukocytosis and thrombocytosis, including JAK inhibitors, will reduce the risk of thrombotic events, progression to PPV-MF and sAML, and consequently, the risk of death. Controlled studies are still needed to determine if current management of PV requires modification based on leukocyte and platelet count. Nonetheless, given that leukocytosis is directly associated with an increased risk of thrombosis [45], a response in leukocyte count is an important aspect of the response to therapy in patients with PV.

Likewise, the use of ruxolitinib to treat patients with PV who have severe symptoms was supported by findings from Study 256 [69] and the RESPONSE study [70••]. In Study 256, symptom analyses included the proportion of patients with a 50 % reduction in symptoms from baseline and those patients with complete resolution of pruritus, night sweats, and bone pain. Clinically meaningful improvements in symptoms were seen within 4 weeks of receiving ruxolitinib and were maintained through week 144 [69]. In RESPONSE, ruxolitinib-treated patients had significant improvements in the 14-item MPN-SAF total symptom score (TSS) compared with those treated with conventional therapy [70••]. Patients receiving ruxolitinib reported improvements in all individual symptoms, especially in those belonging to the cytokine symptom cluster (fatigue, itching, muscle ache, night sweats, and sweating while awake). In contrast, patients treated with standard therapy experienced no change or worsening of their symptoms.

Despite these encouraging results, the phase 3 RELIEF (Randomized Switch Study From Hydroxyurea to Ruxolitinib for RELIEF of Polycythemia Vera Symptoms) study (ClinicalTrials.gov, NCT01632904) showed no significant difference between ruxolitinib and HU for the treatment of persistent symptoms [73]. In RELIEF, patients were randomized to receive ruxolitinib 10 mg twice a day plus placebo (HU; $n = 54$) or HU plus placebo (ruxolitinib; $n = 56$). More patients in the ruxolitinib arm compared with the HU arm (43.4 vs 29.3 %) achieved the study's primary endpoint (a ≥50 % reduction in the MPN-SAF TSS cytokine cluster at week 16). However, despite this positive trend in favor of ruxolitinib, it was not statistically significant ($P = .139$). Although unclear, it is possible that differences in patient populations may have led to such differences in the RELIEF and RESPONSE study results. For example, patients in RESPONSE were HU-resistant or HU-intolerant and were required to have splenomegaly, whereas patients in RELIEF reported symptoms despite treatment with HU and had no splenomegaly. Additionally, differences in trial design (open vs blinded) may have led to the observed differences in study results. Findings from the RESPONSE2 study may help provide answers to some of these unresolved questions.

Other Experimental Strategies

Other JAK inhibitors currently being evaluated in clinical trials for the treatment of MPNs include momelotinib (CYT387) and pacritinib (SB1518). At the time of writing pacritinib has been put onto a full clinical hold by the FDA for bleeding and potential cardiac toxicities. Both have proved encouraging in phase 2 trials, and phase 3 studies in MF are ongoing. Momelotinib is being assessed in a randomized phase 2 study in patients with PV or ET who are JAK-inhibitor naive (Clinicaltrials.gov, NCT01998828); however, its clinical efficacy in PV is unknown. There are currently no studies evaluating pacritinib in this setting.

HDAC inhibitors prevent proliferation of tumor cells by inducing cell-cycle arrest, differentiation, and/or apoptosis, and are therefore desirable candidates in treating malignancies [74]. At least two HDAC inhibitors (vorinostat and givinostat) have been evaluated in patients with PV. However, vorinostat was not well tolerated (44 % of patients discontinued due to adverse events) and is no longer being evaluated [75]. Givinostat has specificity for *JAK2* V617F-mutated cells and was tested in a phase 2 study in patients with *JAK2* V617F-positive PV who were HU-resistant or HU-intolerant ($n = 12$) [76]. Overall, givinostat was well tolerated; 75 % of patients had a reduction in splenomegaly, 54 % had a clinical response after 12 weeks on treatment, and pruritus disappeared in all but one patient. Givinostat was later evaluated in a phase 2 study of patients with PV ($n = 44$) who showed no response when treated with maximum tolerated doses of HU [77]. Patients received givinostat (50 or 100 mg/day) in combination

Table 3 Treatment strategy for patients with inadequately controlled polycythemia vera

Characteristic	First line	Second line
Elevated HCT	Phlebotomy Aspirin	Cytoreductive therapy
Elevated leukocyte and/or platelet counts despite treatment	HU or IFN Anagrelide[a]	Busulfan JAK inhibitor Investigational agent
Burdensome symptoms	Treatment specific for each symptom HU or IFN-α for some symptoms Other therapies (e.g., PUVA, hypnosis)	Switch first line therapies JAK inhibitor Investigational agent
Progressive splenomegaly	HU or IFN-α	JAK inhibitor Investigational agent
Thrombotic event	Indication for cytoreductive treatment, usually HU or IFN-α	Management of thrombotic event and modification of other risk factors
Progression to post-PV myelofibrosis	Treatment according to guidelines for myelofibrosis Consider allogeneic HSCT	
Progression to sAML	Consider intensive chemotherapy and allogeneic HSCT	

HCT hematocrit. *HSCT* hematopoietic stem cell transplantation, *HU* hydroxyurea, *IFN* interferon, *JAK* Janus kinase, *PUVA* psoralen plus ultraviolet A, *PV* polycythemia vera, *sAML* secondary acute myeloid leukemia

[a] For elevated platelet counts only

with HU at the maximum tolerated dose. The combination was well tolerated; only 18 % of patients discontinued treatment: 11 % during the first 12 weeks of treatment and 7 % between weeks 12 and 24. After 12 weeks of treatment, complete or partial response according to ELN criteria was observed in 55 and 50 % of patients receiving 50 or 100 mg givinostat, respectively. Improvements in pruritus were also observed [77]: 58 % of patients with grade 3/4 pruritus showed an improvement or symptom resolution (grade ≤ 1).

Two additional studies with givinostat are currently recruiting. The first study is a dose-finding phase 1/2 nonrandomized study assessing the safety and efficacy in patients with *JAK2* V617F-positive PV (ClinicalTrials.gov, NCT01901432). The primary outcomes will be determination of the maximum tolerated dose, preliminary efficacy, safety, and tolerability. The second study is a multicenter, open-label study evaluating the safety, tolerability, and efficacy of givinostat in patients with PV, ET, or MF who have completed givinostat treatment in a study of chronic MPN or are participating in a compassionate use program with givinostat (ClinicalTrials.gov, NCT01761968). The primary outcomes will be long-term safety and efficacy. Efficacy will be determined based on achievement of complete and partial response rate according to ELN response criteria.

Uncovering the Molecular Underpinnings of PV

Despite such great advancements, there is still more to be accomplished, specifically in further unraveling the genetic basis of PV, preventing thrombosis in patients on treatment, and preventing progression to PPV-MF and sAML. To help achieve these goals, many groups are working on determining the genetic underpinnings of PV, and at least 102 genes outside the *JAK2* pathway that have differential regulation in patients with PV have already been identified [78]. Furthermore, clonal dominance at the progenitor level seems to be present in those patients carrying mutations other than those in *JAK2* and is characteristic of evolution of the disease [79]. In one report of a patient with *TET2*/*JAK2*-positive disease, PEG-IFN-α-2a therapy led to complete molecular response of the *JAK2* clone without reduction of the *TET2* clone [80]. This suggests that other signaling pathways are playing an additional (or complementary) pathogenetic role in PV and that targeted therapies against genes involved in these other pathways, as monotherapies or in combination with ruxolitinib or standard therapies, may aid in the treatment of PV. Additionally, a recent study [81] showed that in patients with PV (and other MPN), the order in which mutations are acquired may have a substantial effect on disease features and the response to therapy. Ortmann and colleagues showed that in patients with PV who carry both *TET2* and *JAK2* mutations, those who acquired *JAK2* mutations first had a more proliferative response to *JAK2* V617F but had *JAK2*-mutant progenitor cells that were more responsive to ruxolitinib in vitro. Interestingly, these patients were also at an increased risk of thrombosis. Further studies will help guide development of molecularly targeted therapies as these become available.

Conclusion

In a proportion of patients with PV, it is important to recognize that their response to current therapies is inadequate and

alternative therapeutic options are needed. We identified these patients as those who, despite treatment, have thrombotic events, elevated HCT, leukocytosis/thrombocytosis, splenomegaly, and burdensome symptoms, and discussed evidence for these being associated with significant events as well as different treatment options available. The discovery of *JAK2* mutations as driver mutations for this disease paved the way for the development of targeted therapies, and now, the first JAK1/JAK2 inhibitor, ruxolitinib, has been approved for the treatment of patients with PV and inadequate response to or intolerance of HU. Its approval may soon lead to the inclusion of JAK inhibitors in the treatment strategy for PV (Table 3). Conventional therapy is less costly than JAK inhibitors, but as discussed, it is not always an optimal treatment. It is clear that for some patients, JAK inhibitors may be more efficacious in alleviating disease burden and improving QOL, and these benefits may ultimately outweigh the cost of therapy. Overall, as new therapies are developed, it will become imperative to identify the appropriate agent for each patient population that is able to prevent thrombotic events without risk of transformation to PPV-MF and sAML.

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Compliance with Ethical Standards

Conflict of Interest Andreas Reiter has participated as an advisory board member, has provided expert testimony, and has received consultancy fees, honoraria, and travel support from Novartis Pharma outside of the submitted work.

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- •• Of major importance

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