blood® Special Report

Moving toward disease modification in polycythemia vera

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Polycythemia vera (PV) belongs to the *BCR-ABL1*–negative myeloproliferative neoplasms and is characterized by activating mutations in *JAK2* and clinically presents with erythrocytosis, variable degrees of systemic and vasomotor symptoms, and an increased risk of both thromboembolic events and progression to myelofibrosis and acute myeloid leukemia (AML). Treatment selection is based on a patient's age and a history of thrombosis in patients with low-risk PV treated with therapeutic phlebotomy and aspirin alone, whereas cytoreductive therapy with either hydroxyurea or interferon alfa (IFN-α) is added for high-risk disease. However, other disease features such as significant disease-related symptoms and splenomegaly, concurrent thrombocytosis and leukocytosis, or intolerance of phlebotomy can constitute an indication for cytoreductive therapy in patients with otherwise low-risk disease. Additionally, recent studies demonstrating the safety and efficacy (ie, reduction in phlebotomy requirements and molecular responses) of ropegylated IFN-α2b support its use for patients with low-risk PV. Additionally, emerging data suggest that early treatment is associated with higher rates of molecular responses, which might eventually enable time-limited therapy. Nonetheless, longer follow-up is needed to assess whether molecular responses associate with clinically meaningful outcome measures such as thrombosis and progression to myelofibrosis or AML. In this article, we provide an overview of the current and evolving treatment landscape of PV and outline our vision for a patient-centered, phlebotomy-free, treatment approach using time-limited, disease-modifying treatment modalities early in the disease course, which could ultimately affect the natural history of the disease.

Introduction

Polycythemia vera (PV) belongs to the *BCR-ABL1*–negative myeloproliferative neoplasms (MPNs) and is characterized by activating mutations in *JAK2* (97% exon 14; 3% exon 12) leading to the proliferation of malignant hematopoietic stem and progenitor cells (HSPCs).[1-3] Clinically, patients with PV present with erythrocytosis, variable degrees of disease-related symptoms (eg, pruritus, night sweats, and fatigue), and an increased risk of both thromboembolic events and progression to myelofibrosis and acute myeloid leukemia (AML).[1,4] The recently published fifth edition of the World Health Organization Classification has reaffirmed the previously established major diagnostic criteria for PV, combining clinical (hemoglobin or hematocrit level >16.5 g/dL or 49%, respectively, in men; and 16 g/dL or 48%, respectively, in women), histopathological (bone marrow findings of trilineage hyperplasia [panmyelosis] and pleomorphic, mature megakaryocytes), and molecular features (presence of *JAK2* ^{V617} or exon 12 mutations).[5-7] A low serum erythropoietin level constitutes a minor criterion for PV diagnosis in the World Health Organization and the recently published International Consensus Classification.[8]

Treatment of patients with PV is individualized based on a patient's age and a history of thrombosis, leading to the dichotomization of patients into those who are at low (no history of thrombosis and age ≤60 years) or high risk of thrombosis (history of thrombosis or age >60 years).[6] Several other disease and patient characteristics have been associated with the risk of thrombosis (Table 1).

All patients with PV independent of risk should receive low-dose aspirin based on randomized, placebo-controlled clinical trial data showing a reduction in the composite risk of nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes (relative risk, 0.40; 95% confidence interval [CI], 0.18-0.91; P = .03).[1,7,24] Additionally, the optimal management of cardiovascular risk factors in collaboration with primary care providers and other medical subspecialists is essential to reduce the risk of thrombotic complications among patients with PV.

In addition to low-dose aspirin, maintaining a hematocrit level <45% is recommended by both US and European management guidelines.[1,7] This recommendation is based on the

Table 1. Risk factors of thrombosis in patients with PV

Risk factor	Effect size	Reference
Age	Age ≥60 y frequently used, but the optimal threshold was unknown; studies using age as continuous variable show increasing risk with older age	9-14
Presence of cardiovascular risk factors (eg, smoking, hypertension, hyperlipidemia, and diabetes mellitus)	Stronger influence on arterial thrombosis (hazard Ratio [HR] 2.0-4.2)	9,13-16
Prior thrombosis	Differences between arterial and venous thrombosis; HR, 2.1-9.7	9,10,12-17
High-risk mutations	Definition and effects size varies across studies; *ASXL1*, *DNMT3A*, *TET2*, and *BCOR/BCORL1* were associated with thrombosis risk in some studies	9,18
JAK2^{V617F} allele burden	*JAK2*^{V617F} VAF >50% is associated with higher risk of venous thrombosis; no association with arterial thrombosis	14-16,19
Leukocytosis	Inconsistent results across studies. WBC count >11 × 10^9/L or >15 × 10^9/L was frequently used as threshold for increased risk of thrombosis. Meta-analysis showing stronger association with arterial thrombosis.	20-23
Treatment	Low-dose aspirin reduces the risk of thrombotic events; hydroxyurea use was associated with a lower risk of thrombosis in nonrandomized study. Limited data available on ruxolitinib and IFN.	10,24-26
Uncontrolled hematocrit	Hematocrit level <45% is associated with a reduced risk of thrombosis	10,14,27
Time from diagnosis	Higher risk during first 3 months since diagnosis	28
Sex	Women had higher rates of venous thromboses; men had more arterial events in the ECLAP study	29
Thrombocytosis	No clear correlation with thrombosis risk	21

WBC, white blood cell.

CYTO-PV study, which demonstrated a reduced risk of major or fatal cardiovascular events in patients with PV, with the hematocrit level maintained strictly below a target of 45% compared with a more liberal target of 45% to 50% (hazard ratio [HR], 3.91; 95% CI, 1.45-10.53; P = .007).[27]

Although US and European guidelines recommend the addition of cytoreductive therapy for patients with high-risk PV, patients with low-risk PV continue to be primarily managed with therapeutic phlebotomy and aspirin alone.[1,7] Of note, frequent phlebotomy or intolerance of phlebotomy, splenomegaly, progressive thrombocytosis, or leukocytosis, and significant disease-related symptoms can constitute an indication for the initiation of cytoreductive therapy for patients with otherwise low-risk PV.[6] However, despite optimal hematocrit control, some patients with low-risk PV continue to experience a reduced quality of life and adverse events related to chronic phlebotomy (eg, iatrogenic iron deficiency) as well as a burden on patients and caregivers because of regular clinic visits.[30,31] Compared with matched controls, the risk of cardiovascular events remains increased among patients with low-risk PV, and almost half of the patients with PV included in a recent health care claims database study had insufficient hematocrit control, with values intermittently reaching >50%.[28,32] Because stable hematocrit control is essential to reduce the risk of thrombotic complications, the intermittent nature of phlebotomy limits its efficacy if hematocrit increases to >45% between treatments.[27,33] Therapeutic phlebotomy also does not address other blood count abnormalities such as leukocytosis, which potentially confers an increased risk of thrombosis, although studies have yielded conflicting results.[20,21] For example, in a recent multicenter, retrospective analysis of patients with PV with persistently elevated leukocyte trajectories had a similar risk of thrombotic events but higher rates of progression to myelofibrosis, myelodysplastic syndrome, or AML.[21] Nonetheless, current guidelines use a leukocyte threshold of >15 × 10^9/L as an indication for cytoreductive therapy.[6]

Additionally, phlebotomy alone does not address the underlying disease process and, as such, does not reduce the risk of long-term events such as progression to myelofibrosis or AML, which are driven by evolution and expansion of the malignant hematopoietic stem cell clone.[34] Thus a new approach to this disease is required.

Currently available cytoreductive therapies for PV

Hydroxyurea

Although current US and European guidelines recommend cytoreductive therapy with hydroxyurea, interferon alfa (IFN-α), or ruxolitinib (second line only for patients with intolerance or inadequate response to hydroxyurea) only for patients at high-risk and selected patients at low-risk, data from a prospective cohort study showed that 66.4% of patients with low-risk PV receive cytoreductive therapy.[1,6,7,30]

Although cytoreductive therapy with hydroxyurea reduces cardiovascular events and remains the most commonly used cytoreductive agent used in PV, it does not reduce the risk of progression to myelofibrosis and AML.[30,35-37] In a propensity-matched cohort study from Italy that compared outcomes in patients with PV treated with phlebotomy alone or hydroxyurea, arterial but not venous thrombosis was significantly reduced among patients treated with hydroxyurea.[38] Especially for younger patients, cytoreduction with hydroxyurea necessitates long-term therapy, which has implications on family planning, an increased risk of cutaneous malignancies (eg, nonmelanoma skin cancer), and concerns related to clonal evolution.[39,40] The specific risk of nonmelanoma skin cancer among patients treated with hydroxyurea is variable, ranging from 0.3% to 27% of patients across studies, but close dermatologic surveillance is needed.[35,41] Additionally, the leukemogenic potential of hydroxyurea remains controversial with most, but not all, studies showing no increased incidence of AML among patients treated with hydroxyurea.[42-44] Nonetheless, alternative agents are needed, especially for younger patients and patients intolerant or refractory to treatment with hydroxyurea.

Interferon

Various formulations of IFN-α have been developed and studied in clinical trials for patients with PV since the 1990s, with a growing body of evidence showing at least noninferiority to hydroxyurea.[36,45-48] Additionally, a subset of patients can achieve a complete molecular remission.[49,50] Table 2 provides an overview of recent phase 2/3 clinical trials of IFN-α in PV. A comprehensive systematic review and meta-analysis was published recently.[47]

Compared with hydroxyurea and phlebotomy, this disease-modifying potential of IFN-α has also been shown to prolong

Table 2. Selected clinical trials of IFN in PV

Author	Intervention	Design	Patient population	Outcomes
Kiladjian et al[51]	peg-IFN-α2a	Single-arm, phase 2 multicenter study	40 patients with PV, aged 18-65 y; no previous treatment, only phlebotomies, or cytoreductive treatment for <2 y	100% ORR (CHR: 95%; PHR: 5%); 72% with complete or partial molecular response
Quintas-Cardama et al[52]	peg-IFN-α2a	Single-arm, open-label, single center, phase 2 study	40 patients with PV; newly diagnosed or previously treated	80% hematologic response rate (CHR 70%); 54% molecular response rate (14% undetectable *JAK2* V617F)
Masarova et al[53]	peg-IFN-α2a	Single-arm, open-label, single center, phase 2 study	43 patients with PV; newly diagnosed or previously treated	84% ORR (CHR: 77%; PHR: 7%); 63% molecular response rate
Yacoub et al[54]	peg-IFN-α2a	Single-arm, phase 2 multicenter study	50 patients with PV; refractory or intolerant to hydroxyurea	60% ORR (22% CR; 38% PR)
Gisslinger et al[36]	ropeg-IFN-α2b vs hydroxyurea	Randomized, open-label, phase 3 trial	257 patients with PV randomized (no, or <3 y of, cytoreductive treatment)	CHR higher with ropeg-IFN at 36 mo (53% vs 38%; *P* = .044); noninferiority of ropeg-IFN for hematologic response and normal spleen size not shown at 12 mo
Gisslinger et al[55]	ropeg-IFN-α2b	Single-arm, open-label, multicenter phase 1/2 study	51 patients with PV	ORR 90% (CR 47%, PR 43%); CMR 21%, PHR 47%
Barbui et al[56]	ropeg-IFN-α2b vs phlebotomy	Randomized phase 2 trial	127 patients with low-risk PV	Higher rates of hematologic response with ropeg-IFN (84% vs 60%; *P* = .0075)
Mascarenhas et al[45]	peg-IFN-α2a vs hydroxyurea	Randomized, open-label, phase 3 trial	87 patients with high-risk PV, randomized; no prior cytoreductive therapy except for up to 3 mo of hydroxyurea	CR rate at 12 mo comparable for IFN vs hydroxyurea (27.9% vs 29.5%; primary end point); ORR 86% vs 68%
Knudsen et al[57]	peg-IFN-α2a or -2b vs hydroxyurea	Randomized, open-label, phase 3 trial	90 patients with PV; newly diagnosed or previously treated	ORR: 68% for hydroxyurea (CHR, 16%; PHR, 53%), 42% (14/33) for IFN-α among patients aged ≤60 y (CHR, 9%; PHR, 33%), and 39% for IFN-α among patients aged >60 y (CHR, 9%; PHR, 30%)

CMR, complete molecular response; ORR, overall response rate; PHR, partial hematologic remission; PMR, partial molecular response.

myelofibrosis-free survival in a large retrospective analysis of patients with low-risk PV, whereas patients with high-risk PV also experienced an overall survival (OS) benefit with IFN-α.[50] However, it is important to note that this was a retrospective study and that there was a significantly higher proportion of patients with high-risk disease in the hydroxyurea group (24% vs 56%; $P < .001$).[50]

Randomized clinical trials demonstrated that both ropegylated IFN-α2b (ropeg-IFN) and pegylated IFN-α2a (peg-IFN-α) are not inferior to hydroxyurea in terms of hematologic response at 12 months.[36,45,57] In the case of the Myeloproliferative Disorders Research Consortium (MPD-RC)-112 study (NCT01259856), this also included bone marrow responses.[45] Importantly, the depth of response to IFN-α deepens over time, with ropeg-IFN having been shown to be superior to hydroxyurea at 36 months in the CONTINUATION-PV study in terms of hematologic control (complete hematologic remission [CHR; defined as a hematocrit level <45% with no phlebotomy for ≥3 months, platelet count <400 × 10^9/L, and leucocyte count <10 × 10^9/L] 53% vs 38%; $P = .044$) and molecular responses (19.6% of patients treated with ropeg-IFN having achieved a *JAK2*^{V617F} variant allele fraction [VAF] of <1% at 60 months compared with only 1.4% in the control group [$P = .0002$]).[36,49] Additionally, the adverse event profile of IFN is distinct from that of hydroxyurea, with liver function test abnormalities, injection site reactions, influenza-like symptoms, and psychiatric symptoms (especially depression) being more common with IFN.[36,45] Limited data support the safety of IFN in pediatric and pregnant patients.[58,59]

For patients who are refractory to or intolerant of hydroxyurea, peg-IFN-α can be an attractive option, with an overall response rate of 60% (22% complete remission [CR]) reported in the MPD-RC–111 phase 2 study (NCT01259856).[54] Interestingly, clinical responses appeared to be independent of a reduction in *JAK2*^{V617F} allele burden.[54] Symptom burden was also significantly improved among responders compared with that among non-responders in a combined analysis of the MPD-RC–111 and MPD-RC–112 trials.[60] Similar response rates have been reported by several other clinical trials using various IFN formulations.[47,51,52,61]

Among patients with low-risk PV, the addition of ropeg-IFN led to higher response rates (hematocrit level <45%) compared with phlebotomy alone in a randomized phase 2 trial (84% vs 60%; $P = .0075$; NCT03003325).[56] Additionally, the total number of phlebotomies per year was lower in the experimental arm (2.8 [95% CI, 2.1-3.5] vs 3.8 [95% CI, 3.1-4.5]; $P = .029$), with 16% of treated patients attaining phlebotomy freedom throughout the 12-month study period.[56] However, rates of treatment-emergent adverse events were higher with ropeg-IFN (78% vs 42%; $P < .0001$).[56] The final results of the trial were recently published, with 81% of patients treated with ropeg-IFN achieving the primary end point of maintaining a median hematocrit level <45% over 12 months in the absence of progressive disease, compared with 51% in the standard group.[62] Additionally, the rate of moderate/severe symptoms was lower in the ropeg-IFN arm compared with in the standard arm after 24 months (33% vs 67%). Patients treated with ropeg-IFN also demonstrated a decrease in the *JAK2*^{V617F} VAF from baseline to 12 and 24 months (−11.9% and −23.1%, respectively), with no changes in the control group. Extended follow-up is necessary to evaluate whether treatment of low-risk PV leads to a reduction in cardiovascular events, progression to myelofibrosis or AML, and ultimately prolongation of OS. Furthermore, it is noteworthy that ropeg-IFN was given at a fixed, low dose of 100 μg every 2 weeks with phlebotomy to maintain a median hematocrit level ≤45% in the Low-PV study, which is different from the PROUD-PV and CONTINUATION-PV studies that used response-adapted doses of ropeg-IFN of up to 500 μg every 2 weeks.[36,62] Whether the fixed, low-dose strategy used in the Low-PV study contributed to the 19% nonresponse rate among patients treated with ropeg-IFN is uncertain, especially because no biomarkers of response were identified in this study. Thus, the best dosing strategy of ropeg-IFN in patients with PV and parameters that should be used for dose titration remain unclear. The latter aspect is currently being explored in the ECLIPSE study (NCT05481151) that randomizes patients with PV to conventional slow titration of ropeg-IFN as per the US Food and Drug Administration label vs an accelerated titration approach. The rationale is to explore whether faster attainment of CHR can induce molecular responses more effectively. A comparison of toxicity profiles will also be informative in determining the balance of clinical effect and tolerability in terms of optimal dosing strategy for ropeg-IFN.

Retrospective data suggest that responses in a subset of patients who discontinue IFN-α are durable, suggesting an elimination (or at least prolonged suppression) of the malignant stem cell clone.[63] This observation would be in line with preclinical studies showing that IFN-α is able to eradicate the disease-initiating *JAK2*^{V617F}-mutant hematopoietic stem cell clone in a murine model.[64] The effect of IFN-α on the HSPC compartment is further supported by data showing that IFN-α targets the human *JAK2*^{V617F}-mutant HSPCs more effectively than mature cells.[65] Furthermore, homozygous *JAK2*^{V617F} clones responded more rapidly than heterozygous clones.[65] Although additional follow-up and prospective validation is needed, this suggests that time-limited therapy in PV might be possible, which is especially attractive for younger patients.

The exact biologic mechanisms by which IFN-α leads to the elimination of the malignant clone remains unknown.[64-66] MPNs, including PV, are characterized by a chronic inflammatory state, which is sustained by the malignant clone itself.[67] IFN-α has been associated with various immunomodulatory functions, enhancing antitumor responses via the activation of B and T cells and natural killer cells and enhanced expression of major histocompatibility complex class I molecules by tumor cells.[46,68,69] Furthermore, IFN-α has antiproliferative and pro-apoptotic effects including inhibition of telomerase activity and downregulation of telomerase reverse transcriptase.[70,71] Finally, gene expression profiles of patients with MPN revealed the deregulation of oxidative stress pathways, which was reversible by treatment with recombinant IFN-α.[72]

The pattern of concurrent mutations could serve as a predictive biomarker and provide additional insights into resistance mechanisms. For example, *TET2* mutations have been associated with lower response rates to IFN-α.[73,74] The impact of other mutations as well as clonal complexity on response likelihood to IFN-α requires additional studies.[73] Additionally, in the DALIAH trial, the emergence of *DNMT3A* mutations was more common among patients treated with IFN-α than that among patients treated with hydroxyurea and was associated with lower rates of CHR.[66] IFN-λ4 diplotype status has been

suggested as a potential biomarker but requires additional validation.[75] Of note, changes in the immune cell repertoire among patients treated with IFN-α did not correlate with hematologic or molecular responses.[68]

Although limited by small numbers, Quintas-Cardama et al showed that patients who failed to achieve a complete molecular remission (defined as undetectable *JAK2*^{V617F} mutation using a polymerase chain reaction assay with 5% sensitivity) had higher rates of clonal evolution than patients who achieved a complete molecular remission (9 of 14 patients vs 0 of 9 patients).[73] However, whether reducing clonal evolution is associated with delaying progression to myelofibrosis or AML requires longer duration of follow-up, and current evidence is insufficient to justify a general recommendation to initiate cytoreductive therapy early for patients with low-risk PV based on preventing clonal evolution alone.

Ruxolitinib

The JAK1/2 inhibitor ruxolitinib has demonstrated superior rates of hematocrit control (60% vs 20%) and CHR (24% vs 9%; $P = .003$) compared with standard of care in a randomized trial of patients with PV and splenomegaly who were phlebotomy dependent and received prior treatment with hydroxyurea.[76] With extended follow-up, responses to ruxolitinib appear sustained, with numerically lower rates of exposure-adjusted thromboembolic events (1.2 per 100 patient-years with ruxolitinib vs 8.2 with best available therapy [BAT]), although OS and progression to myelofibrosis and AML were not improved by treatment with ruxolitinib.[77] A similar open-label phase 3 trial compared ruxolitinib with BAT (hydroxyurea [n = 50%], IFN or peg-IFN [12%], pipobroman [7%], lenalidomide [1%], or no treatment [29%]) among 149 patients with PV without splenomegaly who were intolerant of or resistant to hydroxyurea.[78,79] Hematocrit control by week 28 was achieved in 62% vs 19% of patients treated with ruxolitinib and BAT, respectively (odds ratio, 7.28; 95% CI, 3.43-15.45; $P < .0001$).[79] After 260 weeks of follow-up, 22% of the patients in the ruxolitinib group achieved durable hematocrit control, with a 5-year OS rate of 96% (95% CI, 87-99) vs 91% (95% CI, 80-96) in the BAT group.[78] The role of ruxolitinib in the frontline setting is being explored in the Ruxo-BEAT trial (NCT02577926), with early data showing a significant decrease in both mean hematocrit (45.9%-41.0%; $P = .0003$) and annual number of phlebotomies (4.2-0.96; $P = .0009$).[80] However, a formal comparison with the control arm of BAT is not available. Finally, the phase 2 MAJIC-PV trial randomized 180 patients with PV who were resistant to or intolerant of hydroxyurea to ruxolitinib or BAT and showed a higher CR rate (defined per 2013 European LeukemiaNet criteria) with ruxolitinib than with BAT (43% vs 26%; odds ratio, 2.12; 90% CI, 1.25-3.60; $P = .02$).[25,81] Importantly, event-free survival (EFS; defined as a composite of major thrombosis, hemorrhage, transformation, and death) was superior for patients receiving ruxolitinib (hazard ratio, 0.58; 95% CI, 0.35-0.94; $P = .03$) as was thromboembolic-free survival. EFS was also improved for patients who obtained a CR and/or molecular responses.[25] Interestingly, correlative studies showed a reduction in the clonal burden of *JAK2*^{V617F} in the HSPC compartment of ruxolitinib-treated patients suggesting a direct impact on the malignant stem cell clone, which also correlated with improved EFS.[25] These findings argue for the relevance of molecular responses (defined as a ≥50% reduction in VAF) to the improvement of other clinical outcomes in PV. No trials of ruxolitinib for patients with low-risk PV or as an alternative to phlebotomy have been conducted. However, prior data have shown that *JAK2*^{V617F} allele burden in isolation is an insufficient predictor of thrombosis risk because other disease, patient, and treatment characteristics that interact with *JAK2*^{V617F} allele burden also define the risk of thrombosis.[82,83] Additionally, ruxolitinib was associated with an increased risk of certain adverse events compared with BAT in the MAJIC-PV study, including infections (20 grade 3 or 4 events vs 8 with BAT), squamous cell carcinoma of the skin (11 vs 0 events), metabolic disorders (9 vs 7 grade 3 or 4 events), and herpes zoster infection (27 vs 12 grade 3 or 4 events).[25]

Additional therapeutic options beyond ruxolitinib

For patients who are unable to tolerate or are resistant to second-line treatment with ruxolitinib, enrollment in a clinical trial is preferred. Outside of a clinical trial, treatment with busulfan can be effective, with a CHR rate of 83% reported in a study of 15 patients with advanced PV refractory or intolerant to hydroxyurea.[84] Comparable results have been reported from a multicenter, retrospective study that included 51 patients with MPNs treated with busulfan after hydroxyurea failure, with a rate of complete or partial hematologic remission of 75%.[85] However, treatment with busulfan has been associated with an increased risk of progression to AML in some (but not all) studies; therefore, this agent is best used for older patients in need of better blood count control.[84,86,87] Other treatment options such as pipobroman or radioactive phosphorus should not be used based on limited efficacy and leukemogenic potential.[6,88]

Combination therapies

Because inflammation is an important contributor to the pathogenesis of PV, combination treatment with ruxolitinib and IFN-α could have synergistic effects, given the immunomodulatory properties of both agents.[46] Additionally, the antiinflammatory effects of ruxolitinib could increase the tolerability to IFN-α and reduce rates of early discontinuation. Early-stage clinical trials evaluating the safety and efficacy of combination therapy with ruxolitinib and peg-IFN-α have recently been reported. In a single-arm phase 2 trial (EudraCT2013-003295-12) of ruxolitinib and peg-IFN-α that enrolled 32 patients with PV (94% with prior peg-IFN-α exposure), 31% achieved either a complete (9%) or partial (22%) hematologic remission.[89] The mean reduction in *JAK2*^{V617F} VAF was numerically greater among patients who were previously intolerant to peg-IFN-α or were IFN-α naive compared with patients who were previously refractory (61%, 65%, and 34%, respectively).[89] It remains to be seen whether early combination therapy for patients with newly diagnosed PV can be more effective at eliminating the malignant clone and reducing the risk of thrombosis or disease progression. Preliminary data from 25 patients with newly diagnosed PV (19 at high risk and 6 at low risk) enrolled in an ongoing single-arm trial of ruxolitinib + peg-IFN-α showed attainment of CHR in all 25 patients, with significant reductions in *JAK2*^{V617F} VAF (median of 47% [95% CI, 35-59] at baseline to 6% [95% CI, 3-12] after 24 months; 4 patients achieved a *JAK2*^{V617F} VAF < 1%).[90] There was 1 case of acute myocardial infarction but no other thromboembolic events. Additionally, 1 patient progressed to myelofibrosis 10 months after starting treatment.[90]

The combination of ruxolitinib with either hydroxyurea or IFN-α for patients with newly diagnosed high-risk PV is also being studied in a randomized, open-label phase 3 trial (NCT04116502).

Investigational agents

Selected ongoing clinical trials of novel agents in PV are summarized in Table 3. In an ongoing phase 2 trial, rusfertide, a hepcidin mimetic, reduced the hematocrit level to <45% without the need for phlebotomy in all 16 patients who were phlebotomy naive and who received treatment.[91] Among patients with insufficient hematocrit control despite phlebotomy with and without cytoreductive therapy, rusfertide substantially reduced the mean number of phlebotomies from 4.63 in the 28 weeks before enrollment to 0.43 with treatment.[92] Although these results are highly encouraging and the sustained and constant hematocrit control with rusfertide might lead to a lower risk of thrombotic events than with intermittent phlebotomy, larger studies with longer duration of follow-up are needed. Additionally, it is important to note that rusfertide is unlikely to lead to a reduction in leukocyte count or to sustained molecular remissions based on its mechanism of action that appears to be primarily centered on iron metabolism.[21,22,92] The randomized, placebo-controlled phase 3 VERIFY trial (NCT05210790) is ongoing and should provide additional insights into the efficacy and safety profile of rusfertide in a broader patient population.[93]

Murine double-minute 2 (MDM2) is a key negative regulator of the tumor suppressor protein p53 that inhibits p53 function and has been shown to be upregulated in PV stem and progenitor cells, making it a potential therapeutic target.[94,95] Preclinical

Table 3. Ongoing clinical trials in PV

Agent (mechanism of action)	NCT (trial name if available)	Phase	Patient population
Ruxolitinib (JAK1/2 inhibitor)	NCT04644211	2	Low-risk PV (no history of thrombosis and age <60 y)
TGR-1202 (PI3Kδ inhibitor) + ruxolitinib	NCT02493530	1	Patients with MF, MDS/MPN, or PV resistant to hydroxyurea
Ruxolitinib vs BAT	NCT02577926 (RUXO-BEAT)	2	High-risk PV or ET; no prior cytoreductive therapy permitted
Ruxolitinib in combination with either hydroxyurea or IFN-α	NCT04116502 (MITHRIDATE)	3	High-risk PV defined as WBC count >11 × 10^9/L + at least 1 of the following: age >60 y, prior thrombosis or hemorrhage, or platelet count >1000 × 10^9/L
ropeg-IFN-α2b	NCT05485948	2	Patients with PV with intolerance or resistance to hydroxyurea
Phlebotomy + aspirin w/wo pegylated proline-IFN-α2b (AOP2014)	NCT03003325 (Low-PV)	2	Patients with low-risk PV; prior cytoreductive therapy was not permitted
ropeg-IFN-α2b	NCT04655092	3	Long-term extension study in patients with PV previously treated with ropeg-IFN-α2b
ropeg-IFN-α2b (randomized between various dosing regimens)	NCT05481151 (ECLIPSE PV)	3	Patients with PV; no specifications regarding phlebotomy or prior cytoreductive therapy
Rusfertide (PTG-300-08; hepcidin mimetic)	NCT04767802 (PACIFIC)	2	Newly diagnosed PV with a baseline hematocrit level >48%
Rusfertide vs placebo	NCT05210790 (VERIFY)	3	Patients with PV who are phlebotomy dependent
Sapablursen (antisense oligonucleotide targeting transmembrane protease serine 6 (TMPRSS6) mRNA)	NCT05143957	2	Patients with PV who are phlebotomy dependent
PPMX-T003 (monoclonal antibody against transferrin receptor 1)	NCT05074550	1	Patients with PV who are phlebotomy dependent; cytoreductive therapy not permitted
IMG-7289 (LSD1 inhibitor)	NCT04262141	2	Patients with ET or PV with intolerance or resistance to at least 1 line of prior therapy
Givinostat (HDAC inhibitor)	NCT01761968	2	Long-term extension study in patients with *JAK2* V617F-mutant MPNs previously treated with givinostat
Rivaroxaban or apixaban vs aspirin	NCT05198960 (AVAJAK)	3	Patients with high-risk PV or ET (aged >60 y or prior thrombotic event) with *JAK2* V617F mutation

ET, essential thrombocythemia; HDAC, histone deacetylase; LSD1, lysine-specific demethylase 1; MDS, myelodysplastic syndrome; MF, myelofibrosis; mRNA, messenger RNA; NCT, National Clinical Trials number; WBC, white blood cell; w/wo, with or without.

Table 4. Proposed definition of clinical trial end points and biomarkers suggestive of disease-modifying activity

	Proposed definition	Advantages	Disadvantages and controversies	Ref
Time-to-event end points				
Thrombosis-free survival	Time from randomization (or study entry) to the date of the first major thrombosis (defined as stroke, acute coronary syndrome, transient ischemic attack, pulmonary embolism, abdominal thrombosis, deep-vein thrombosis, or peripheral arterial thrombosis; should be reported as composite and separately for arterial vs venous events) or death from any cause	Clinically relevant outcome because thrombotic events drive morbidity and mortality in PV Objective and measurable Treatment (ie, hematocrit control) has been shown to reduce risk of thrombosis	Low event rates require extended study duration Variable definition across clinical trials	4,25,27
Myelofibrosis-free survival	Time from randomization (or study entry) to the date of progression to myelofibrosis or death from any cause	Association with OS and symptom burden Objective assessment possible	Low event rates Rates depend on other factors (eg, comutations) Hematologic response may not correlate with progression to myelofibrosis	50,100
Leukemia-free survival	Time from randomization (or study entry) to the date of progression to AML or myelodysplastic syndrome or death from any cause	Association with OS Objective assessment possible	Depend on other factors (eg, comutations) Low event rates	25
OS	Time from randomization (or study entry) to the date of death from any cause	Objective assessment Improving OS as the most robust end point	Low event rates	50
Composite end points				
Progression-free survival	Time from randomization (or study entry) to the date of progression to myelofibrosis, AML or myelodysplastic syndrome, or death from any cause	Association with OS Objective assessment possible	Variable definitions across clinical trials	25
EFS	Time from randomization (or study entry) to the date of first major thrombotic event or progression to myelofibrosis, AML or myelodysplastic syndrome, or death from any cause	Captures all major contributors to PV-related morbidity and mortality Earlier readout than individual components Objective assessment possible	Variable definitions across clinical trials Not every component of the composite end point has the same implications	25
Clinical trial end points				
Possibly reflecting disease-modifying effects				
Complete hematologic response	Defined per ELN 2013 response criteria as durable (≥12 wk) peripheral blood count remission, defined as hematocrit level <45% without phlebotomies; platelet count ≤400 × 10^9/L, WBC count <10 × 10^9/L	Most commonly used end point in clinical trials Normalization of WBC and platelet count potentially reduces risk of progression to myelofibrosis and thrombosis Shorter time to trial readout	Controversy whether hematologic response correlates with risk of thrombosis	17,21,36,81
Complete response per ELN2013 criteria	Defined per ELN2013 response criteria	Composite of clinical and pathologic response Normalization of CBC associated with survival and thrombosis risk	Requires repeat bone marrow biopsy for response	27,81

CBC, complete blood count; ELN2013, European LeukemiaNet 2013; EQL-5D, European Quality of Life–5 dimensions; MPN-SAF, Myeloproliferative Neoplasm–Symptom Assessment Form; Ref, reference; WBC, white blood cell.

Table 4 (continued)

	Proposed definition	Advantages	Disadvantages and controversies	Ref
Molecular response rate	Reduction in *JAK2*^{V617F} allele	JAK2 allele burden is correlated with risk of venous thrombosis Molecular responses are associated with lower risk of disease progression Early readout	Requires additional prospective validation and standardization Uncertainty regarding optimal threshold Differences between arterial and venous events The threshold by which *JAK2*^{V617F} VAF needs to be reduced to associate with clinical benefit remains to be defined	16,25
Clonal evolution	Acquisition of additional somatic mutations or cytogenetic abnormalities on serial bone marrow evaluations	Early readout Acquisition of certain comutations associated with higher risk of progression	Requires additional prospective validation and standardization	100,101
Symptom control	Defined per ELN2013 response criteria as reduction in MPN-SAF by ≥10 points	Patient centered Higher symptom burden is associated with quality of life in other domains and rates of depression	Unclear correlation with OS, thrombosis, and disease progression Uncertainty regarding optimal assessment tool (EQL-5D, MPN-SAF) The optimal threshold for symptom improvement is unclear	45,81,102-104

CBC, complete blood count; ELN2013, European LeukemiaNet 2013; EQL-5D, European Quality of Life–5 dimensions; MPN-SAF, Myeloproliferative Neoplasm–Symptom Assessment Form; Ref, reference; WBC, white blood cell.

data also suggest synergy of combination treatment with MDM2 inhibition and IFN.[94] In a recently completed phase 2 study (NCT03287245) of the MDM2 inhibitor idasanutlin that enrolled 27 patients with PV who were phlebotomy dependent and hydroxyurea resistant/intolerant, 56% and 50% achieved hematocrit control and CHR, respectively.[96] Response rates were comparable among patients who were ruxolitinib naive and those previously exposed to ruxolitinib (55% vs 60%).[96] Additionally, there was a median reduction in *JAK2*^{V617F} allele burden by 76%, which was associated with hematologic responses.[96] However, gastrointestinal adverse events were common (nausea [93%; 11% grade ≥3], diarrhea [78%], and vomiting [41%]) and 41% of enrolled patients discontinued treatment early.[96] Analysis of patient samples also revealed a transient expansion of *TP53*-mutant clones during treatment with idasanutlin, which was reversible with treatment discontinuation and not associated with disease progression.[97] Nonetheless, this phenomena will require attention in future studies.

Future directions

We believe that increased awareness and a better understanding of disease modification across MPNs will be crucial for drug development and may ultimately change the treatment paradigm for patients. Such efforts are already underway to begin defining what constitutes disease modification in myelofibrosis.[98,99] Similar to myelofibrosis, disease modification in PV likely includes several aspects such as cytokine level modulations and hematologic, molecular, and histopathologic parameters and their relationship to objective and clinically relevant clinical outcomes (eg, thrombotic events, progression to myelofibrosis and AML, and OS). Ultimately, the goals of treatment should be refined and focused on allowing patients the best chance to live a normal lifespan free of the sequelae of the disease. With this premise in mind, we have summarized clinical trial end points and surrogate markers for disease-modifying effects of investigational therapies (Table 4).

Given the absence of a disease-modifying treatment effect, the associated burden on patients and caregivers, and the development of novel, alternative approaches, the role of phlebotomy as the primary and exclusive treatment for patients with low-risk PV is diminishing. Additionally, the use of hydroxyurea as the default choice for cytoreductive therapy is debatable, especially with the approval of novel IFN-α formulations that are better tolerated and could offer an attractive option for a broad range of patients with low- or high-risk disease. Increasing evidence suggests that IFN-α has the potential to change the natural history of PV with deep (and sometimes durable) molecular remissions in a subset of patients that may translate into an improved myelofibrosis-free survival and OS.[50] Whether this could enable time-limited therapy (ie, treatment for a finite period of time followed by treatment discontinuation and active surveillance after a predefined response threshold is met) and what patient and disease characteristics may predict response to IFN-α remain to be determined with the execution of prospective trials with sufficient follow-up. In the case of younger patients, the benefits of IFN-α could include improvement in the quality of life with a reduction in the time spent in the health care office for receiving therapeutic phlebotomies and the potential long-term toxicities associated with hydroxyurea.[31] However, we would like to emphasize that the

current evidence does not support the general implementation of such an early, IFN-α–based intervention strategy for all patients with low-risk PV. Longer follow-up is necessary to evaluate whether early intervention and the high rates of hematologic and molecular responses seen with IFN-α–based therapies lead to a reduction in clinically relevant long-term outcomes such as thrombotic events and progression to myelofibrosis and AML. Especially, because treatment with IFN-α can be associated with adverse events in patients who would otherwise not receive any medication, selecting patients at high risk of disease progression, for example based on molecular features, can lead to a more favorable risk-to-benefit ratio.[100] Finally, the health-economic implications of this paradigm shift are unclear and warrant further study.

With the increasing availability of molecular testing, a paradigm shift away from indefinite therapy to a time-limited treatment approach might become possible. Such time-limited treatment could be based on predefined clinical and molecular parameters similar to the milestones established for treatment discontinuation in chronic myeloid leukemia based on quantitative BCR-ABL1 transcript assessments.[105] For example, Kiladjian et al suggested potential criteria for treatment discontinuation (*JAK2*^{V617F} VAF < 10%; sustained CHR for ≥2 years; and no disease progression, thromboembolic events, or worsening of disease-related signs or symptoms over the entire treatment period).[49] However, prospective validation of these criteria is needed.

Furthermore, novel prospective studies with end points such as progression-free survival and OS are needed to advance the treatment of patients with PV. Such trials will likely require many years to complete, and a concerted and coordinated investment of time and resources on the part of investigators and the pharmaceutical industry will be required. In parallel, the search for surrogate markers of progression-free survival and OS using existing data sets should be pursued and integrated into prospective clinical investigations (Table 4).

Although normalization of complete blood count (CBC) parameters remains the cornerstone of PV management and should be considered an essential end point in clinical trials of PV,[27] we would argue that it is time to incorporate molecular responses more prominently in the response criteria; data from recent trials demonstrating improved EFS in patients treated with ruxolitinib achieving molecular responses as well as older data showing a lower rate of progression to myelofibrosis among patients with PV with low *JAK2*^{V617F} allele burden support such considerations.[25,83,106] Because hematologic and molecular responses are frequently correlated among patients treated with IFN but not those treated with hydroxyurea,[25,36,62,83] we would suggest reporting both hematologic and molecular end points in clinical trials, with an emphasis on standardization of assessment and reporting. Additional prospective data are needed to determine what the optimal depth of molecular response is, because *JAK2*^{V617F} is among the most common mutations encountered in individuals with clonal hematopoiesis.[107] With increasingly sophisticated techniques it will also be possible to disentangle the HSPC compartment from other cell types harboring *JAK2*^{V617F}. However, molecular responses require time to achieve and can deepen over time, as exemplified by the multiple studies with IFN, which is an important consideration for clinical trial design.

Finally, it will be essential to standardize testing of *JAK2*^{V617F} in terms of which assay to use, timing of assessment, and whether peripheral blood or bone marrow should be analyzed. This harmonization effort across the research community in collaboration with regulatory agencies will be essential to establish *JAK2*^{V617F} allele burden as a molecular biomarker of response.

In summary, our current treatment approaches are inadequate to help patients with PV live the longest and best lives possible. Available therapies such as IFN provide an opportunity for patients with PV to achieve deep molecular responses as a surrogate for disease burden reduction, and the integration of mutational testing into the management of PV should allow us to delineate minimal residual disease states. Cooperative group approaches with academia, community-based practitioners, industry partners, and regulatory agencies will need to focus resources and energy into efficiently developing the next generation of prospective clinical trials with end points such as EFS and OS and embedded molecular correlates that not only identify predictive biomarkers for response but also surrogate biomarkers for remission and functional cure.

Authorship

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Footnote

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