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## Management of hydroxyurea resistant or intolerant polycythemia vera

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### ABSTRACT

Polycythemia vera is a Philadelphia negative myeloproliferative neoplasm characterized by erythrocytosis in which the major cause of morbidity and mortality is thrombosis. Aspirin and hematocrit reduction by venesection or cytoreductive therapy are at the cornerstone of management. First line cytoreductive therapy in high-risk patients is hydroxyurea; however, its use is associated with toxicities and resistance in a significant proportion of patients. In a disease with a long overall survival with appropriate treatment, it is imperative that other treatment options do not accelerate the risk of progression to acute leukemia. The following review will appraise the evidence of interferon, ruxolitinib, and other agents in management of hydroxyurea resistant or intolerant polycythemia vera.

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### Introduction

Polycythemia vera (PV) is a BCR-ABL negative myeloproliferative neoplasm characterized by erythrocytosis in peripheral blood and pan-myelosis in the bone marrow with a detectable driver mutation in the *JAK2* gene in over 98% of cases [1].

The disease is associated with a high risk of thrombosis [2], and current management is largely aimed at reducing the risk of thrombotic events and associated mortality. The cornerstones of treatment are hematocrit control and aspirin use. In the landmark “Cytoreductive Therapy in Polycythemia Vera” (CYTO-PV) study, patients randomized to maintain a hematocrit below 45% had a lower rate of cardiovascular death and thrombosis compared to patients who were randomized to a more liberal target hematocrit range of 45–50% [3]. The European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) study showed a reduced risk of arterial and venous thrombosis with low dose aspirin in PV patients [4]. Current guidelines stratify patients into low-risk and high-risk groups based on age and a history of thrombosis [1,5,6]. The European LeukaemiaNet (ELN) consortium has used age  $\geq 60$  and/or history of thrombosis to define their high-risk group, and the British Society of Hematology have used a higher threshold of age  $\geq 65$

and/or history of thrombosis for the same [5,6]. All patients should be managed with aspirin (except in the presence of acquired von Willebrand’s disease associated with thrombocytosis), strict control of modifiable cardiovascular risk factors, and maintenance of hematocrit below 45%.

While patients with low risk PV may be managed with phlebotomies to achieve hematocrit control, those with high risk PV should instead receive cytoreductive therapy, and the first-line treatment recommendation for cytoreductive therapy across most guidelines is hydroxyurea. However, treatment with hydroxyurea is associated with intolerance and resistance in 15–24% of patients [7,8]. The most troubling toxicities include fever, painful leg and mucocutaneous ulcers, and pneumonitis [7,9,10]. Resistance to hydroxyurea includes ongoing phlebotomy requirements to keep hematocrit  $< 45\%$ , uncontrolled myeloproliferation (white cell count  $> 10 \times 10^9/L$  or platelet count  $> 400 \times 10^9/L$ ) and/or failure to reduce splenomegaly by 50%, despite 3 months of therapy with  $\geq 2g$  per day of hydroxyurea [9]. Hematological toxicity (absolute neutrophil count  $< 1.0 \times 10^9/L$  or platelet count  $< 50 \times 10^9/L$ ) at the lowest dose that maintains clinico-hematological response is also included in the definition of hydroxyurea resistance

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**Table 1.** European LeukaemiaNet 2013 criteria for response assessment in Polycythemia Vera.

Complete Remission	Partial Remission
Durable <sup>a</sup> resolution of disease-related signs including palpable hepatosplenomegaly, large symptoms improvement <sup>b</sup> AND	Durable <sup>a</sup> resolution of disease-related signs including palpable hepatosplenomegaly, large symptoms improvement <sup>b</sup> AND
Durable <sup>a</sup> peripheral blood count remission, defined as hematocrit lower than 45% without phlebotomies; platelet count $\leq 400 \times 10^9/L$ , WBC count $< 10 \times 10^9/L$ , AND	Durable <sup>a</sup> peripheral blood count remission, defined as hematocrit lower than 45% without phlebotomies; platelet count $\leq 400 \times 10^9/L$ , WBC count $< 10 \times 10^9/L$ , AND
Without progressive disease, and absence of any hemorrhagic or thrombotic event, AND	Without progressive disease, and absence of any hemorrhagic or thrombotic event, AND
Bone marrow histological remission defined as the presence of age-adjusted normocellularity and disappearance of trilinear hyperplasia, and absence of $>$ grade 1 reticulin fibrosis	Without bone marrow histological remission defined as persistence of trilinear hyperplasia

No response: any response that does not satisfy partial remission. Progressive disease: transformation into post-PV myelofibrosis, myelodysplastic syndrome or acute leukemia. Adapted with permission from Barosi et al. [12].

<sup>a</sup>Lasting at least 12 weeks.

<sup>b</sup>Large symptom improvement ( $\geq 10$ -point decrease) in Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) cytokine total symptom score (TSS-C).

[9]. Furthermore, it is well known that patients with hydroxyurea resistance are at an increased risk of transformation to myelofibrosis and acute myeloid leukemia [7].

This review will summarize the evidence behind therapeutic alternatives to hydroxyurea in PV.

### Response assessment

To enable standardization of response assessment and ease of comparison between clinical trials, ELN response criteria were developed in 2009 [11] and revised in 2013 [12]. Complete remission (CR) by ELN 2009 response criteria is defined as hematocrit less than 45% in the absence of phlebotomies, white cell count (WCC)  $\leq 10 \times 10^9/L$ , platelet count  $\leq 400 \times 10^9/L$ , and absence of splenomegaly. Partial remission (PR) is achieved when the target hematocrit of less than 45% is reached, or in the absence of achieving the target hematocrit fulfilling all of the other clinical criteria defined above. The revised criteria in 2013 include bone marrow histological remission as an additional criterion for demonstration of CR (Table 1).

### Interferon

Over the last three decades, several studies have confirmed that interferon alpha is effective in controlling myeloproliferation in Philadelphia negative myeloproliferative neoplasms [13]. However, due to its toxicity profile and high discontinuation rates, it was not previously used first line. Since the discovery of *JAK2V617F* mutation and the observation that interferon targets the malignant clone, interest has been renewed in this drug [14]. Following the development of a PEGylated formulation over 15 years ago, several phase 2 and phase 3 studies in PV have been conducted. Interferon alpha mediates responses in myeloproliferative neoplasms through anti-

proliferative, pro-apoptotic, antiangiogenic, and immunomodulatory effects [15–17].

While the majority of studies discussed below have shown that PEGylated interferon alpha is efficacious in inducing hematological and spleen responses, it is associated with a high discontinuation rate owing to toxicities [13]. The most common toxicities include pruritus, arthralgia, fatigue, headache, diarrhea, influenza-like illness, vertigo, psychiatric adverse events, and grade 1/2 elevation of liver enzymes [13,18–23]. Other less common toxicities include autoimmune phenomena with a high representation of thyroid disease, circulating ANA and rash [13].

In the relapsed or refractory setting, if hydroxyurea has been used as first line, the recommendation as per current guidelines is to use PEGylated interferon alpha as second line therapy, and vice versa [6].

The longest follow up data of an early phase 2 clinical trial of pegylated interferon alpha 2a in PV was published by Masarova et al. in 2017 [21]. In this study, 43 patients with newly diagnosed or previously hydroxyurea treated PV received weekly PEGylated interferon alpha 2a and were followed up for a median of nearly 7 years. CR was defined as normalization of peripheral blood counts and spleen size, and PR was defined as at least 50% reduction in phlebotomies or spleen size. The ORR and CR in PV patients was 80% and 70%, respectively. Patients with CR held their response for a median of 65 months (over 5 years), and at last median follow up of 83 months (nearly 7 years), 30% of all patients still had a hematological response. Molecular response was seen in 60% of patients, comprising a complete molecular remission (CMR) or undetectable *JAK2 V617F* in 20% and partial molecular response (PMR) or  $>50\%$  reduction in *JAK2 V617F* allele burden in 40% of patients [21]. Similar to other studies, 22% of patients discontinued treatment due to toxicities.

Of the several published trials of PEGylated interferon in PV, the “Myeloproliferative Disorders Research Consortium (MPD-RC)-111” study is the only one to have excluded newly diagnosed and/or hydroxyurea untreated patients [23]. The MPD-RC-111 study was a phase 2 study that assessed response to PEGylated interferon alpha 2a, as defined by ELN 2009 response criteria, in hydroxyurea intolerant or refractory patients. The overall response rate was 60%, but the discontinuation rate due to adverse events was 18%, and only 50% of patients completed 2 years of therapy.

Gisslinger et al. conducted one of the few studies with ropegylated interferon alpha 2b which has a longer half-life than PEGylated interferon alpha 2a and may be administered fortnightly [19]. Patients with newly diagnosed and hydroxyurea treated PV were enrolled in this phase 1/2 multicenter study and treated with ropegylated interferon alpha 2b in a dose escalation phase and a dose expansion phase. In this study, 38 of 51 patients completed 50 weeks of treatment. Although the overall response at one year was 82% with a CR rate of 26%, only patients who completed treatment were included in the final analysis.

Following on from this, the PROUD PV study recruited 257 patients in a large, phase 3 randomized controlled trial that compared ropegylated interferon alpha 2b with hydroxyurea in the upfront setting [24]. The primary outcome was peripheral blood count remission and spleen response at the end of 12 months, and patients that continued past 12 months were recruited into the CONTINUATION PV (CONTI-PV) study [25]. At the end of 12 months, the primary outcome was achieved in 21% of the ropegylated interferon alpha 2b group as compared to 28% of the hydroxyurea, thereby proving non-inferiority of ropegylated interferon alpha 2b in the upfront setting at 12 months. However, when followed up to 36 months in the CONTI-PV study, statistically superior CR rates were seen in the ropegylated interferon alpha 2b group (53% versus 38%;  $p$  value = 0.04). Additionally, a superior molecular response (66% versus 27%,  $p$  value < 0.0001) was seen in the ropegylated interferon alpha 2b group compared to the hydroxyurea group at 36 months. When compared to the pegylated interferon alpha 2a trials, there was a lower rate of discontinuation of ropegylated interferon alpha 2b at 8%, and this is thought to be due to optimized protein engineering and reduced nonspecific pegylation of this formulation, thereby improving efficacy and tolerability of the drug.

The results of the 5-year analysis of this study showed that hematocrit response continued to be maintained in 81.8% versus 63.2% ( $p$  = 0.01) of

patients randomized to receive ropegylated interferon alpha 2b versus hydroxyurea [26]. The superior molecular response was sustained in the ropeg-treated patients compared to hydroxyurea treated patients (69.1% versus 21.6%;  $p$  < 0.0001). In the ropeg-treated group, one patient progressed to myelofibrosis; and in the hydroxyurea treated group, there were two cases of progression to myelofibrosis and two cases of transformation to acute myeloid leukemia.

Roppegylated interferon alpha 2b is also being investigated in the low-PV study, and the results of a pre-planned interim analysis were presented at the 25th European Hematology Association annual congress [27]. In this phase 2 study, low-risk PV patients were randomized to receive conventional therapy of monthly phlebotomy and daily aspirin or fortnightly subcutaneous ropegylated interferon alpha 2b in addition to conventional therapy. The interim analysis was performed on 100 patients who completed 12 months of therapy, and 84% versus 60% ( $p$  value 0.038) of patients randomized to ropegylated interferon alpha 2b versus conventional therapy maintained the target hematocrit of less than 45%. Given the overwhelming efficacy, recruitment of new patients into the trial was stopped and existing patients will be followed up to 2 years. There was no difference in grade  $\geq 3$  events in both groups.

Across these studies, it is clear that interferon alpha results in a reduction in the malignant clone compared with hydroxyurea, but there remain high rates of discontinuation due to toxicity, particularly with pegylated interferon alpha 2a. The reduction in allele burden is associated with higher rates of CR, but the significance of this in prolonging overall survival and reducing rates of transformation remains to be seen. When compared to hydroxyurea in the upfront setting, PEGylated interferon alpha is non-inferior in efficacy, but less well tolerated [24]. In the relapsed refractory setting, patients who are heavily pretreated have a lower molecular response and a shorter duration of response. Up to 50% of patients in this group are likely to discontinue PEGylated interferon alpha due to toxicities or progressive disease within 2 years of treatment. Regardless, due to its property of targeting the malignant clone, PEGylated interferon alpha is a popular second-line therapeutic choice in polycythemia vera (Table 2).

### Ruxolitinib

Ruxolitinib is a potent inhibitor of JAK1 and JAK2, and in clinical trials in myelofibrosis ruxolitinib

**Table 2.** Characteristics, outcomes, and adverse events in clinical trials with PEGylated interferon alpha 2a in hydroxyurea treated patients.

Author	Year	Patients	N	Treatment and schedule	Outcome	Outcome definition	Adverse events
Kiladjian et al. [20]	2008	PV patients with less than 2 years of previous cytoreductive therapy	37	Weekly peg-IFN alpha-2a: 90–180 mcg	ORR: 100% (CR: 94.6%; PR: 5.4%) MR: 72.4%	CR: Peripheral blood count remission and no splenomegaly PR: 50% reduction in phlebotomies	24.3% discontinuation rate
Quintás-Cardama et al. [46]	2009	Newly diagnosed and hydroxyurea treated patients	43	Weekly peg-IFN alpha-2a: 90–450 mcg	ORR: 80% (CR: 70%, PR: 10%) MR: 60%	2009 ELN response criteria	22% discontinuation rate
Yacoub et al. [23]	2019	Hydroxyurea refractory or intolerant patients	50	Weekly peg-rIFN alpha-2a: 45–180 mcg	ORR: 60% (CR: 22%, PR: 38%)	2009 ELN response criteria	18% discontinuation rate

CR: complete remission; ELN: European LeukaemiaNet; MR: molecular response; ORR: overall response rate; peg-IFN: pegylated interferon; PR: partial response; ropeg-IFN: ropegylated interferon.

demonstrated significant improvement in symptom severity and splenomegaly [28]. While JAK1 plays an important role in the signaling of many proinflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor alpha (TNF $\alpha$ ), JAK2 is important for signaling of hematopoietic growth factors such as erythropoietin, GM-CSF, thrombopoietin, IL-3, IL-5, growth hormone, and prolactin [29]. Treatment with ruxolitinib has been proven to reduce multiple fibrogenic, pro-inflammatory and angiogenic growth factors [30].

The first study with ruxolitinib in hydroxyurea refractory or intolerant PV patients included 34 patients who received the drug in a dose escalation phase, followed by a dose expansion phase at a dose of 10 mg of ruxolitinib twice a day [31]. The primary end points of CR and PR, as defined by modified ELN 2009 criteria were achieved by 59% and 38% of the cohort respectively. Infection with herpes zoster was seen in 14.7% of the cohort. Only 23.5% of the cohort achieved a PMR or >50% reduction in *JAKV617F* allele burden, and no patients achieved a CMR. This study showed that despite a good response in hematological parameters and splenomegaly to ruxolitinib, the molecular response did not mirror the clinical response.

RESPONSE was the first, large phase 3 study of 222 hydroxyurea resistant or intolerant PV patients with splenomegaly who were randomized to either ruxolitinib or standard treatment [32]. Standard treatment included hydroxyurea at the highest tolerated dose, interferon, anagrelide, lenalidomide, or thalidomide. The primary end point was percentage of patients who achieved hematocrit control (defined as sustained hematocrit  $\leq$  45% without phlebotomies between week 8 and week and 32) and at least 35% reduction in spleen size. At 32 weeks, 22.7% of patients in the ruxolitinib arm and 0.9% of patients in the standard treatment arm met the primary end point ( $p < 0.0001$ ); and 60% of patients in the ruxolitinib arm achieved hematocrit control as compared to 18.8% of patients in the standard treatment arm. Furthermore, 49% of patients randomized to ruxolitinib achieved >50% reduction in total symptom score as compared to 5% of patients in the standard arm. Thromboembolic events occurred at a reduced rate of 1.8 events per 100 person years in the ruxolitinib arm, compared to 8.2 events per 100 person years in the standard treatment arm. However, it should be noted that majority of patients in the standard treatment arm received hydroxyurea. Furthermore, the median reduction in JAK2 V617F allele burden at the end of 80 weeks was only 22%. The RESPONSE trial showed that ruxolitinib

was clinically efficacious in polycythemia vera patients with splenomegaly, but not as effective at inducing a robust molecular response.

RESPONSE 2 was a phase 3 randomized controlled study that differed from RESPONSE by including hydroxyurea refractory or intolerant PV patients without splenomegaly, and the primary endpoint was percentage of patients who had sustained hematocrit control in the absence of phlebotomy between week 8 and week 28 [33]. Ruxolitinib was commenced at 10 mg BD and increased to 25 mg BD to meet the primary endpoint. A total of 149 patients were recruited in the study, and ultimately, 64% of those randomized to ruxolitinib and 19% of those randomized to BAT met the primary end point ( $p$  value  $< 0.0001$ ). Once again, the mean *JAK2* V617F VAF reduction at week 80 in the ruxolitinib arm was modest, at 9.7% [34]. Similar to previous studies, infection rates with herpes zoster were seen at an increased rate in the ruxolitinib arm.

The Expanded Treatment Protocol (ETP) study was another phase 3b, single arm, multicenter study of ruxolitinib in hydroxyurea refractory or intolerant PV patients in which the primary endpoint was safety data [35]. Hematocrit control was achieved in 45.3% and normalization of peripheral blood counts was achieved in 18% of patients. Amongst all patients with splenomegaly at baseline, 86.7% had at least 50% reduction in spleen size. Adverse events leading to drug discontinuation were seen in 8.7% of the cohort. The most common hematological adverse event was all grade anemia seen in 31.8% of patients. Thrombocytosis was seen in 10% of patients, and most likely attributable to disease, as opposed to being a drug effect. The most common non-hematological adverse events were headache, constipation, diarrhea and fatigue. The rate of herpes zoster infection was 1.8 per 100 person years.

The results of the MAJIC PV study were recently presented in abstract form, and had superior results in CR rates with ruxolitinib as compared to previous similar studies [36]. In this study, 182 hydroxyurea resistant or intolerant patients with PV were randomized to either ruxolitinib or BAT and the primary endpoint was CR by ELN 2009 criteria at 1 year. This occurred in 49.5% of patients randomized to ruxolitinib compared to 27% of patients randomized to BAT ( $p$  value of 0.0009). It is important to note that a higher rate of CR was also seen in the BAT arm when compared with previous studies.

The RELIEF study is the only double blinded randomized controlled trial involving ruxolitinib in PV to date [37]. The study included 110 patients with PV

on a stable dose of hydroxyurea with controlled blood counts but ongoing symptoms, as defined by an MPN-SAF TSS-C score of 8 or more, who were randomized to either ruxolitinib or to continue hydroxyurea. The primary endpoint of  $\geq 50\%$  reduction in the MPN-SAF TSS-C score at 16 weeks was met by 46.4% of patients randomized to ruxolitinib and 29.6% of patients randomized to hydroxyurea. This difference was not statistically significant. Among individual symptoms, statistical superiority with ruxolitinib was seen for the improvement of pruritus alone. The double-blinded approach of this trial highlighted the significant placebo effect seen in the group of patients randomized to continue hydroxyurea.

In summary, ruxolitinib appears to be very effective for hematocrit control and spleen response. The RESPONSE study showed a clear benefit in reduction of venous thromboembolism events. There is less consistency with regard to *JAK2* V617F allele burden reduction as compared to interferon. The major adverse events across most studies were treatment-related anemia and herpes zoster infection. While there are no current guidelines on routine antimicrobial prophylaxis in patients on ruxolitinib, anti-viral prophylaxis should be considered in patients at high risk of zoster reactivation (Table 3).

### Combination therapy with PEGylated interferon and ruxolitinib

The results of the recent COMBI study have been eagerly awaited as this is the first trial that combines ruxolitinib with PEGylated interferon alpha [38]. This was considered to be an attractive combination due to the potential synergistic effect of the two drugs, and possible mitigation of interferon side effects by concomitant ruxolitinib use. The COMBI study was a single arm phase 2 study of PEGylated interferon alpha and ruxolitinib in PV and myelofibrosis patients. PV patients with active disease were eligible for recruitment if they had an ECOG of  $< 3$ , WCC  $\geq 1.5 \times 10^9/L$  and platelet count  $\geq 100 \times 10^9/L$  in the absence of severe comorbidities. The primary endpoint was efficacy at 12 months and 2013 ELN criteria (bone marrow assessment included) was used to assess efficacy.

In this study, 32 PV patients with a median age of 57 were recruited and 66% of them had high-risk disease. At 12 months the overall response rate in PV patients was 31% and included 3 patients with CR and 7 patients with PR as defined by 2013 ELN criteria. In addition, three other patients achieved bone marrow

**Table 3.** Characteristics, outcomes, and adverse events in clinical trials with ruxolitinib in polycythemia vera.

Author (Clinical trial)	Year	Population	Number of patients	Treatment(s)	Outcome in ruxolitinib arm versus outcome in comparator arm <sup>c</sup> (p value) <sup>c</sup>	Outcome definition	Adverse events in ruxolitinib versus comparator arms
Verstovsek et al. [31]	2014	Hydroxyurea refractory or intolerant PV patients	34	Ruxolitinib	ORR: 97%; CR: 59%; PR: 38%; MR: 23.5%	CR: Modified 2009 ELN criteria <sup>a</sup> PR: Hct < 45% MR: >50% reduction in VAF	Herpes Zoster in 14.7% DR: 5.9%
Verstovsek et al. [32] (RESPONSE)	2016	Hydroxyurea refractory or intolerant PV patients with splenomegaly	222	Ruxolitinib versus BAT	CR: 22.7% versus 0.9% (p < 0.001) MR: 34.4% versus 1.2% Hct < 45%: 60% versus 18.8% CR: 64% versus 19% (p < 0.0001)	CR: Hct < 45% <sup>d</sup> MR: >50% reduction in VAF	Herpes Zoster in 6.4% versus 0% DR: 4.5%
Passamonti et al. [33] (RESPONSE 2)	2017	Hydroxyurea refractory or intolerant patients	149	Ruxolitinib versus BAT		CR: Hct < 45% <sup>d</sup>	Grade ≥ 3 anemia in 14.3% versus 3.7% Herpes Zoster 3.8 per 100 person years versus none Herpes zoster: 1.8 per 100 person years. DR: 8.7%
Foltz et al. [35] (ETP)	2018	Hydroxyurea refractory or intolerant patients	161	Ruxolitinib	Hct < 45% <sup>d</sup> : 45.3% PBCr: 18% Spleen response <sup>b</sup> : 86.7% CR: 49.5% versus 27%	PBCr: Hct < 45%; platelet count ≤ 400 × 10 <sup>9</sup> /L, WBC count < 10 × 10 <sup>9</sup> /L 2009 ELN response criteria	Grade ≥ 3 anemia: 6.5% versus 1.1% Grade ≥ 3 infections: 10.8% versus 5.6%
Harrison et al. [36] (MAJIC PV)	2018	Hydroxyurea refractory or intolerant PV patients	182	Ruxolitinib versus BAT		2009 ELN response criteria	Grade ≥ 3 anemia: 6.5% versus 1.1% Grade ≥ 3 infections: 10.8% versus 5.6%
Mesa et al. [37] (RELIEF)	2017	Stable polycythemia vera with ongoing symptoms on hydroxyurea	110	Ruxolitinib versus hydroxyurea (double blinded)	Endpoint: 46.4% versus 29.6% (p = 0.139)	Endpoint: ≥50% reduction in MPN SAF TSS-C score	Herpes zoster: 1.9% versus 0% DR: 7.4% versus 1.8%

BAT: best available treatment; CR: complete response; DR: discontinuation rate; Hct: hematocrit; MR: molecular response; ORR: overall response rate; VAF: variant allele frequency; PBCr: peripheral blood count remission; PV: polycythemia vera.

<sup>a</sup>Modified ELN criteria: peripheral blood count remission, no pruritus, no palpable splenomegaly.

<sup>b</sup>Spleen response: ≥35% reduction in spleen size in patients with splenomegaly.

<sup>c</sup>When applicable or available.

<sup>d</sup>In the absence of phlebotomy, and lasting at least 12 weeks.

hematological remission who did not fulfill certain other criteria for CR. At 2 years from recruitment, at least 60% of the PV cohort achieved CR. A majority of the cohort had a sustained CR for more than 3 months during the 2 years of follow up. The median time to CR was 1 month.

A molecular response (>50% reduction in allele burden) was seen in 25% of PV patients, and patients with a molecular response were more likely to achieve a clinico-hematological response. In the entire cohort, the median JAK2V617F allele burden reduced from 47% at baseline to 12% at the end of 2 years.

At the end of 2 years, two patients had episodes of thrombosis and one patient had progressed to post PV myelofibrosis. Unfortunately, discontinuation of PEGylated interferon occurred in 31% of trial patients, and this statistic is consistent with rates associated with PEGylated interferon monotherapy.

It should be noted that response was assessed by ELN 2013 criteria and, therefore, the results are not directly comparable to previous studies with single agent interferon or ruxolitinib. In addition, a lower dose of interferon was used in this study and as seen in previous studies the clinical and molecular effects of interferon may occur beyond 2 years of follow up. Encouragingly, several patients had bone marrow histological remission and reductions in JAK2V617F burden allele. However, while only 6% of PV patients dropped out of the study, 25% of PV patients discontinued PEGylated interferon. Regardless, the COMBI study has showed us that combination therapy with PEGylated interferon and ruxolitinib is certainly safe and efficacious in PV patients.

### Novel targeted therapies

The results of several trials evaluating novel investigational agents are eagerly awaited. MDM2 is a negative regulator of TP53, and studies have shown that it is upregulated in PV hematopoietic stem cells. Idasanutlin is an oral MDM2 antagonist and a phase 1 study that enrolled 11 high risk PV patients reported an overall response of 58% [39]. Results from the initial part of the phase 2 idasanutlin study in hydroxyurea resistant or intolerant PV patients were discussed at the 62nd American Society of Hematology (ASH) annual meeting and exposition [40]. Unfortunately, the study has been terminated by the sponsor despite promising results in efficacy due to frequent gastro-intestinal toxicities, in particular nausea that was refractory to anti-emetics. Response assessments were conducted in 16 patients who

**Table 4.** Ongoing clinical trials in polycythemia vera with novel agents.

Molecule	Mechanism of action	Phase	Clinicaltrials.gov ID
Umbralisib	PI3K delta inhibitor	2	NCT02493530
PTG-300	Hepcidin mimetic	2	NCT04057040
KRT 232	MDM2 inhibitor	2	NCT03662126

completed 32 weeks on trial. Normalization of peripheral blood counts was seen in 50% of these patients. A spleen response of >35% volume reduction was seen in 33% of patients, but unfortunately this was not sustained until 32 weeks in most, and only one patient met the composite end point of spleen and hematocrit response at the end of 32 weeks.

Histone deacetylase inhibitors have also demonstrated efficacy in PV. While one study with vorinostat showed high discontinuation rates due to non-hematological toxicities [41], givinostat has been better tolerated.

The most recent study of givinostat in PV was a 2 part Phase Ib/IIa study with a dose escalation phase and a dose expansion phase [42]. Response was assessed by clinico-hematological parameters of the ELN criteria at 3 and 6 months. In the first phase, 12 patients were recruited, and the maximum tolerated dose was determined to be 100 mg BD of givinostat. In the second phase of the study, 35 patients were treated with 100 mg BD of givinostat.

While ORR is reported to be 70–80%, only two and four patients achieved CR in the first and second phases, respectively. Nevertheless, the response in individual hematological parameters was notable with hematocrit, WCC, and platelet response seen in 77.4%, 90%, and 74% of patients, respectively, in the second phase of the study. An improvement in disease-related symptoms was reported, but this was most pronounced in pruritis. The number of patients with severe pruritis decreased from 45.2% before treatment to 6.5% at the end of 3 and 6 months. Diarrhea and thrombocytopenia were the most common all grade adverse events and occurred in >45% of all patients.

Of the other novel agents being investigated in phase 2 clinical trials (Table 4), hepcidin mimetic PTG-300 (ClinTrials.gov Identifier: NCT04057040) is a first in class molecule with a novel mechanism of action. Hepcidin is a master regulator of iron metabolism, and is in turn regulated by erythroferrone secreted by erythroid precursors [43]. Iron deficiency is common in PV and exacerbated by phlebotomy. Increased erythroferrone and decreased hepcidin expression is seen in PV patients [43]. In a pre-clinical murine model of PV, hepcidin agonists normalized hematocrit levels and reduced splenomegaly [44]. Early data from the PTG-



300-04 study was recently presented at the 62nd ASH annual meeting and exposition [45]. This is a phase 2 study of subcutaneously administered hepcidin mimetic PTG-300 in therapeutic phlebotomy dependent PV patients. Of eight patients who completed 3 months of treatment, only two patients had transient elevations of hematocrit above 45%, one of whom required a therapeutic phlebotomy. Erythrocyte numbers decreased in all but two patients, and ferritin progressively increased in patients. With only grade 1/2 injection site reactions seen in 3 patients, these results are very promising.

## Conclusion

Despite the development of new therapeutic options for PV, it must be reiterated that no treatment has been proven to alter the natural history of the disease or reduce the rate of transformation to myelofibrosis and/or acute myeloid leukemia. The endpoints of trials reported over the last decade are largely surrogates of disease activity, and long-term follow up results for leukemia-free and myelofibrosis-free survival are awaited.

However, there is clearly a protective effect of appropriate management on thrombosis risk. Therefore, it is vitally important that all patients are treated with aspirin and that hematocrit is maintained below 45% either by phlebotomy or cytoreductive therapy. It is crucial that the risk of transformation is not increased through the use of leukemogenic agents. While interferon has been shown to suppress the malignant clone, long-term data regarding its potential effect on rates of transformation to acute myeloid leukemia and myelofibrosis are awaited. There is a lack of long-term safety and efficacy data regarding the use of ruxolitinib. Therefore, its use in PV, which has a longer median overall survival than myelofibrosis, is less certain. Outside the setting of a clinical trial, it should be reserved for older PV patients, those with rapidly progressive/refractory disease or for those with refractory symptoms.

Meanwhile, a range of novel therapies are on the horizon, with new hopes being pinned on HDAC inhibitors and hepcidin mimetics.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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