Review

Current management strategies for polycythemia vera and essential thrombocythemia

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ABSTRACT

Polycythemia vera (PV) and essential thrombocythemia (ET) are myeloproliferative neoplasms characterized by increased rate of cardiovascular events, a varying burden of symptoms, and an intrinsic risk of evolution to secondary forms of myelofibrosis and acute leukemia; however, survival is only modestly reduced in most instances. In the last few years, following the description of driver mutations in *JAK2*, *MPL* and *CALR*, the diagnostic criteria for PV and ET were revised, making the identification of very early stages feasible. Scores for identifying patients at different risk of thrombosis were refined, and they largely guide therapeutic decisions. Treatment is therefore mainly focused on reduction of thrombosis risk, control of myeloproliferation, improvement of symptomatic burden, and management of disease-associated complications. New drugs recently entered the clinical arena, with the promise to improve overall patients' management. However, evidence of a disease-modifying potential is largely missing and represents a still unmet clinical need.

1. Introduction

Polycythemia vera (PV) and essential thrombocythemia (ET) are the two most frequent among the Philadelphia chromosome-negative myeloproliferative neoplasms (MPN). Their clinical course is smooth and uneventful in a substantial proportion of the patients, while conversely it may be highly symptomatic, resulting in impairment of quality of life, and characterized by progressive worsening, responsible for reduction of survival, in the remaining others.

In this manuscript, we will outline what are the most relevant aspects of the management of patients with PV and ET, largely based on recent guidelines and consensus recommendations for diagnosis, risk stratification and treatment, that inform our own daily practice. These were developed by different organizations, networks and experts' groups, and include the World Health Organization [1,2], the IWG-MRT International Working Group for Myeloproliferative neoplasms Research and Treatment (IWG-MRT) [3], the European Leukemia Net (ELN) [4–6], the European Society of Medical Oncology (ESMO) [7] and the National Comprehensive Cancer Network (NCCN) [8]. However, we acknowledge that different experts' opinions and practice patterns exist, as outlined in experts' position papers [9] as well as in guidelines/consensus papers from national scientific societies such as the British Society for Hematology [10,11], the Austrian Society of

Hematology [12], the Canadian MPN group [13], to name a few; discussion of such differences are outside the scope of this review, and the interested reader is directly referred to the original publication.

2. Criteria for making diagnosis

To make diagnosis of PV, we suggest to follow the criteria lastly revised in 2016 by the WHO; they are listed in Table 1 [1]. Compared to the previous classification of 2008, the criteria were substantially modified following the description of an early phase of the disease, initially called "masked PV" [14–16]. Changes consisted in: i) lowering of the hemoglobin threshold to 16.5 g/dL (hematocrit, 49%) and 16.0 g/dL (hematocrit, 48%) for men and women, respectively, (corresponding values in the 2008 version were 18.5 g/dL and 16.5 g/dL); ii) the inclusion of bone marrow (BM) biopsy as a major diagnostic criterion, and iii) the dismissal of the erythropoietin-independent erythroid colonies (EEC) test as a diagnostic tool since, although highly specific for *JAK2*V617F mutated, erythropoietin-independent erythroid progenitors [17], it suffers from being technically demanding, expensive and its availability being limited to a few academic laboratories. More than 95% of patients with abnormally increased hemoglobin/hematocrit levels due to an underlying MPN are positive for the *JAK2*V617F mutation; the distribution of individual mutant variant

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Table 1
Diagnostic criteria for the chronic phase of PV and ET according to the 2016 WHO classification).

	Polycythemia Vera	Essential thrombocythemia
Major	1. Hemoglobin > 16.5 g/dL in men/Hemoglobin > 16.0 g/dL in women or, Hematocrit > 49% in men/ Hematocrit > 48% in women or, increased red cell mass (RCM)[1]. 2. BM biopsy[2] showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature, megakaryocytes (differences in size). 3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation.	1. Platelet count ≥450 × 10^9/L. 2. BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers. 3. Not meeting WHO criteria for *BCR-ABL1* CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms. 4. Presence of *JAK2*, *CALR*, or *MPL* mutation[3].
Minor	Subnormal serum erythropoietin level.	Presence of a clonal marker or absence of evidence for reactive thrombocytosis.
Criteria required for diagnosis	All 3 major or the first 2 major and the minor criterion	All 4 major criteria or the first 3 major and the minor criteria

[1] More than 25% above mean normal predicted value.

[2] BM biopsy may not be required in cases with sustained absolute erythrocytosis defined as Hb levels 18.5 g/dL in men (hematocrit, 55.5%) or 16.5 g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present.

[3] In the absence of any of the 3 driver mutations, the search for the most frequent accompanying mutations (eg, *ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1*) is of help in determining the clonal nature of the disease.

allele fraction (VAF) is ample, ranging from 0.1% to virtually 100%, with a median level around 50% [18]. Homozygosity of the *JAK2*V617F mutation is therefore common, and is considered as a hallmark of PV as compared to ET [19,20], where homozygosity is infrequent and the median VAF in the overall population of patients is around 20–30%. In cases of erythrocytosis where the V617F mutation is absent, search for mutations in *JAK2* exon 12 is recommended [21,22]; these mutations are heterogeneous indels and require a greater deal of technical efforts for their accurate detection, complicated by their often very low VAF [23]. Subnormal to normal serum erythropoietin (EPO) levels are included as a minor diagnostic criterion in the WHO classification; however, in at least 15% of the patients with *JAK2*-mutated PV, serum EPO levels at diagnosis fall within the normal range [24]. The WHO criteria do not mandate BM biopsy for patients with hemoglobin levels greater than 18.5 g/dL (men) and 16.5 g/dL (women), since these levels are considered strong surrogate evidence of pathologically expanded red cell mass (by definition, this corresponds to a > 25% increase of the expected, individual-adjusted, red cell mass value). However, BM biopsy is recommended to assess BM fibrosis, particularly in younger subjects who have long projected disease duration, since a grade ≥1 fibrosis at diagnosis has been associated with increased risk of transformation to PPV-MF and an overall worse outcome [25]. The most direct approach to determine an increased red cell mass would be concurrent isotope determination of red cell mass and plasma volume (with labelled albumin) [9,26]; while this has to be accredited as the "gold standard", routine performance of the test is hampered by its intrinsic difficulties and cost, owing to the use of radioisotope and the need of accredited facilities, and the number of institutions currently performing this test is very limited.

As regards diagnosis of ET, we similarly follow the definitions included in the 2016 WHO criteria (Table 1), that include: i) confirmed thrombocytosis in excess of 450 × 10^9 platelets/L, ii) a BM biopsy showing proliferation of mature, normal appearing megakaryocytes, iii) exclusion of other mimicking conditions characterized by thrombocytosis, *in primis BCR-ABL1* mutated chronic myelogenous leukemia, and iv) presence of one driver mutation (*JAK2*V617F, *CALR*, *MPL*) or, if absent, evidence of a clonal marker or accurate exclusion of reactive thrombocytosis. The *JAK2*V617F mutation is found in about 60% of the patients with ET, followed by mutation of *MPL*W515 in < 5% and mutation of *CALR* in 20–25%. Histopathology is mandatory also to exclude other myeloid disorders with thrombocytosis (see Table 1), in particular a myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, that manifests with anemia and ≥15% ring sideroblasts in the BM, thrombocytosis, and a *SF3B1*

mutation (that in > 70% of cases is associated with *JAK2*V617F mutation). One very important change introduced by the latest WHO classification is the recognition of precise criteria to distinguish ET from prefibrotic or early forms of MF (prePMF). Although a reticulin fibrosis not greater than grade 1 can be found in both ET and prePMF, the two diseases have to distinguished by careful analysis of BM biopsy. In the latter condition, megakaryocytes are often found in clusters, display abnormal maturation with hyperchromatic and irregularly folded nuclei, and are surrounded by increased cellularity with granulocytic proliferation and often decreased erythropoiesis; this pattern contrasts with the overall normal/reduced cellularity and the appearance of megakaryocytes in ET, that are enlarged and mature with hyperlobulated nuclei [1]. The prognostic implication of an accurate differentiation of ET from prePMF is supported by several studies [27,28]. In the largest cohort of almost 700 cases, median survival was 30.2 years for patients with ET compared to 14.7 years for those with prePMF, accounting for a hazard ratio of 2.7 (95%CI, 1.9–3.7) of prePMF versus ET [28].

Several studies indicate that 10–15% of patients with a WHO-based diagnosis of ET do not harbor typical MPN-associated driver mutations, and are referred to as "triple negative". Conceivably, these subjects may actually have other clonal, molecularly undefined, forms of thrombocytosis or, as shown recently, a minority harbor non-canonical mutation of *JAK2* and *MPL* [29,30]; precise understanding of these forms of thrombocytosis is currently missing, and how they should be classified and managed is an unmet question, complicated by the fact that in some cases the clonal nature of thrombocytosis cannot be demonstrated. It is important to realize that these triple-negative forms of thrombocytosis appear to have a very benign course [31], opposite to triple-negative patients with myelofibrosis who suffer from a substantially reduced survival compared to other mutation group, in particular patients with *CALR*-type 1 mutation [28].

Approximately 10 to 15% of patients with ET and PV transform to myelofibrosis after a median of 15 years of disease. Diagnosis of transformation to PPV-MF and PET-MF requires fulfilling the criteria developed, through consensus procedure, by the IWG-MRT; they are outlined in Table 2 [3]. Diagnosis is based on a combination of the following findings: development of a grade 2–3 BM fibrosis; worsening of anemia (or, in case of PV, sustained loss of phlebotomy and/or chemotherapy requirements to maintain target hematocrit); appearance or worsening of pre-existing mild splenomegaly: development of constitutional symptoms; and, appearance of a leucoerythroblastic picture in peripheral blood. In case of patients with preexisting ET, increased lactate dehydrogenase (LDH) plasma levels represent an additional

Table 2
Diagnostic criteria for post-PV and post-ET myelofibrosis according to the IWG-MRT consensus.[1]

	Post-polycythemia vera myelofibrosis	Post-essential thrombocythemia myelofibrosis
Required	1. Documentation of a previous diagnosis of PV as defined by the WHO criteria. 2. Bone marrow fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale)[1].	1 Documentation of a previous diagnosis of ET (WHO criteria). 2 BM fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale)[1].
Additional	1. Anemia[2] or sustained loss of requirement of either phlebotomy (in the absence of cytoreductive therapy) or cytoreductive treatment for erythrocytosis. 2. A leukoerythroblastic peripheral blood picture. 3. Increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm (distance of tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly. 4. Development of ≥1 of three constitutional symptoms: > 10% weight loss in 6 months, night sweats, unexplained fever (> 37.5 °C).	1. Anemia[2] and a ≥ 2mgml decrease from baseline Hb level. 2. A leukoerythroblastic peripheral blood picture. 3. Increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly. 4. Increased LDH (above reference level). 5. Development of ≥1 of three constitutional symptoms: > 10% weight loss in 6 months, night sweats, unexplained fever (> 37.5 °C).
Criteria required for diagnosis	All 2 required criteria and at least 2 additional criteria	All 2 required criteria and at least 2 additional criteria

[1] Grade 2–3 according to the European classification or Grade 3–4 according to the standard classification.
[2] Below the reference range for appropriate age, sex, gender and altitude considerations.

accessory criterion, a finding whose value has been questioned, however [32].

3. Criteria for risk-stratifying patients for treatment decisions

There is no definite proof yet that any of the treatments employed for patients with PV and ET has the potential to cure the disease. As discussed later, interferon was reported to induce complete molecular remission of *JAK2*V617F mutation in at least some cases, with much more experience for PV than ET patients, but the persistence of clones harboring other myeloid gene mutations speaks, in theory, against the concept of "cure", an interpretation, however, complicated by the recent description of clonal, age-related, hematopoiesis of undetermined potential (CHIP) [33]. In addition to the absence of demonstration of a cure with currently available drugs, we also lack evidence that therapies may appreciably delay progression to PPV-MF and PET-MF or prevent leukemic transformation. Therefore, the end-points of treatment are currently represented by control of disease-associated symptoms and reduction of risks of morbidity and mortality associated with cardiovascular complications (thrombosis and hemorrhage), as indicated in the ELN recommendations [6,34], ESMO guidelines [7] and the NCC guidelines [8]. On this ground, and in order to tailor as much as possible the individual treatment and balance at best the therapeutic needs and goals and the therapy-related side-effects, the criteria employed for risk stratification of patients with PV and ET are based on combination of variables that are credited to be predictive of thrombosis risk in this population. This contrasts with scores developed for patients with primary myelofibrosis [35,36], where it is the projected duration of survival that drives therapeutic decisions, especially as regards the indication of the only curative option represented by stem cell transplantation [37]; a score specifically developed for patients with PPV-MF and PET-MF has recently been developed [38]. However, clinical [39] and molecular [40] variables associated with shortened survival in PV and ET were also reported, although their use is of minimal utility, if at all, in clinical practice.

Older age (> 60 years) and previous episodes of major cardiovascular events are the two classic variables used to stratify patients with PV and ET in a conventionally defined "high-risk" category -patients presenting either of the 2 variables- and a "low-risk" category -none of the above. Generic cardiovascular risk factors, particularly hypertension [41], smoking and leukocytosis, contribute, as it might be intuitive, to the overall risk of thrombosis, but they are not formally included yet in risk scoring system. In PV, hypertension was found to increase the annual rate of thrombosis from 0.85% patients/year to 2.05% patients/year and from 2.4% patients/year to 3.65% patients/year in the low- and high-risk conventional category, respectively [41].

As regarded leukocytosis, in a time-dependent multivariable analysis of the European Collaborative study of Low-dose Aspirin in PV (ECLAP) cohort (n = 1638 patients) [42], a leukocyte count > 15 × 10^9/L increased the hazard ratio of major thrombosis of 1.71 fold (95%CI, 1.1–2.6) compared to patients with ≤10 × 10^9/L leukocytes [43]. Furthermore, in the CYTO-PV trial, a prospective randomized trial, that enrolled 365 patients with PV [44], aimed at testing the effects of intensity of cytoreduction on thrombosis rate, a leukocyte count ≥11 × 10^9/L accounted for an HR for thrombosis of 3.90 (95%CI, 1.24–12.03) compared to the reference category (leukocytes < 7 × 10^9/L) [45]. Similarly, in patients with ET, contribution of leukocytosis to the overall risk of thrombosis is supported by several studies [46–48]. A recent meta-analysis, that included 41 studies involving more than 30,000 patients, reported a relative risk (RR) of any degree of leukocytosis for all thrombotic events of 1.34 (95%CI, 1.06–1.66) for PV and 1.65 (95%CI, 1.43–1.91) for ET; when considering type of thrombosis, leukocytosis remained associated with arterial thrombosis (RR 1.45; 95%CI, 1.13–1.86) unlike venous thrombosis (RR 1.14; 95%CI, 0.65–1.98) when PV and ET patients were considered together [49]. Although a discrete level of leukocytosis that could be formally considered as a risk factor for thrombosis has not been formally defined yet (a task that would require a prospective, controlled setting), the available evidence is strongly supportive of a causative relation effect [50]. However, in the above mentioned meta-analysis, based on the distribution of cut-off values of leukocytosis, weighted according to each study sample size, a prognostically meaningful WBC cut-off of 12.4 × 10^9/L was identified in the combined ET and PV population, while values for individual diseases were 11.0 × 10^9/L and 15.0 × 10^9/L for ET and PV, respectively [49]. On the other hand, it is noteworthy that no evidence supports the platelet count as a risk factor for thrombosis in PV [51] and ET. In particular, in the prospective Primary Thrombocythemia (PT-1) study in patients with ET, having a platelet count outside normal range at any time point during the follow-up did not increase the risk of thrombosis, while conversely it was associated with bleeding episodes [52]. In fact, extensive thrombocytosis is considered a risk factor for bleeding owing to a concomitant condition of acquired von Willebrand disease [53,54].

In patients with ET, an improvement in patients' risk stratification compared to the standard 2-tiered score is represented by the revised IPSET (International Prognostics Score System)-thrombosis) score [55,56]. The score received independent validation [57,58]. The variables included in the IPSET score, after adjustment for sex, hemoglobin level, leukocyte and platelet count, use of hydroxyurea and aspirin, were the following: age > 60 years (HR 1.50; 95%CI, 1.0–2.25), generic cardiovascular risk factors (HR 1.56; 95%CI, 1.02–2.36), previous thrombosis (HR 1.93; 95%CI, 1.27–2.91) and *JAK2*V617F

mutation (HR 2.04; 95%CI, 1.19–3.48). Revised IPSET considers four categories of risk: a very low-risk (no risk variable is present), low-risk (presence of *JAK2*V617F mutation), intermediate-risk (age > 60 years, no thrombosis history, *JAK2* un-mutated) and high-risk category (history of thrombosis and/or age > 60 years and *JAK2*V617F mutation) [56]. Although patients with ET harboring *CALR* mutation have reduced risk of thrombosis compared to those who were *JAK2*V617F and *MPL*W515 mutated [59,60], the introduction of *CALR* genotype among the IPSET variables did not modify appreciably the predictive power of the score [61]. However, taking into an account the very low rate of thrombotic events of WHO-ET *CALR* mutated patients [59], how these subjects should be managed as regards antithrombotic and cytoreductive therapy is still a matter of debate (see below).

4. Goals of therapy and assessment of response to therapy

The goals of therapy for patients with PV and ET, according to the ELN recommendations [34], are the prevention of first occurrence and/or recurrence of thrombotic and bleeding complications while minimizing the risk of progression to MF or AL, the effective control of systemic symptoms, and the appropriate management of complications and risk situations [34]. Criteria for standardized assessment of response to treatment were developed in 2013 by the ELN and IWG-MRT conjunctly, and include the improvement of blood cell counts, splenomegaly, symptoms and histology. Overall, there are different levels of coded response (complete response, partial response, no response, progressive disease), and, of note, molecular response is not required for adjudicating complete or partial response [62]. However, the aim of these criteria was to create uniformity in reporting the results of clinical trials, rather than fixing precise response criteria useful in conventional setting to adapt treatment thereafter. Furthermore, the importance of attaining a complete hematologic response for long-term benefits (cardiovascular events, hematological progression, survival) has not been addressed yet in controlled studies, and remains largely debated [63].

5. Treatment of patients with PV

The cornerstone of treatment for patients with PV is based on two recommendations, that are evidence-based since they derive from prospective randomized trials. The first recommendation concerns the control of increased red cell mass, that in terms of clinical practice translates into the recommendation to maintain a steady hematocrit level to < 45%. In the phase 3 randomized CYTO-PV trial, PV with patients were randomized to be "cytoreducted" to maintain a hematocrit level at < 45% with phlebotomy alone, for conventionally defined low-risk patients, or/plus cytoreductive agents for high-risk patients (the drug used in > 80% was hydroxyurea). Results of the study indicated that patients randomized to the < 45% hematocrit arm had 4-fold less major thrombotic events compared to patients randomized to the arm allowing a more relaxed hematocrit control, that is a hematocrit level between 45 and 50% [44]. The ultimate aim of phlebotomy is to induce a condition of chronic iron deficiency, key to adequate disease control; however, iron supplementation may be indicated occasionally, in those few cases manifesting clear iron-deficiency associated symptoms, and should be performed very cautiously since a few days of oral iron in a PV patient may result in rapid increase of hematocrit [64]. Asthenia, one disease-associated manifestation commonly referred by PV patients, has to be attributed to the systemic inflammatory condition rather than purely to iron deficiency, as also supported by the observation that JAK2 inhibitors have rapid activity on the symptoms, largely before iron deficiency can be ameliorated [65]. Considering that the red cell mass in normal women is lower than in man, based on radioisotopic studies of red cell mass and plasma volume, some experts indicate that the < 45% hematocrit level may not be appropriate for women, and point to a sex-adjusted hematocrit level

at < 42% as the most adequate; however, while this approach may be well adopted in clinical practice, a study that demonstrates the superiority of one versus the other hematocrit target has not been accomplished yet [64].

The second recommendation concerns the use of low-dose aspirin (81 to 100 mg/day) as primary anti-thrombotic prophylaxis for all patients with PV and who do not have contraindications to aspirin, independent of risk categories. In a placebo-controlled study, the ECLAP (European Collaboration on Low-dose Aspirin in PV) trial, low-dose aspirin reduced the combined risk of nonfatal cardio-embolic events (myocardial infarction, stroke, pulmonary embolism, major venous thrombosis) or cardiovascular death by 60% (relative risk, 0.40; 95% confidence interval, 0.18 to 0.91); there was no significant increase of major bleeding episodes in the experimental arm compared to placebo (relative risk, 1.62, 95% confidence interval 0.27 to 9.71) [42]. Whether these results should be re-interpreted on the basis of recent studies failing to demonstrate risk-balanced effectiveness of aspirin for primary prophylaxis in large cohorts of normal individual without prior history of atherosclerotic cardiovascular disease (reviewed in a meta-analysis [66]) represents an important research issue for future studies; in the meanwhile, considering that PV patients constitute a population of subjects at intrinsically high risk of cardiovascular events, our attitude is to base therapeutic approach on the evidences produced in the ECLAP study. It may also be that a more aggressive control of hematocrit level in low-risk patients without additional generic cardiovascular risk factors makes aspirin prophylaxis less beneficial, counterbalancing the risk of hemorrhagic complications; this might be an additional experimental question to address.

According to current guidelines, use of cytotoxic drugs is indicated in patients conventionally defined at high risk, and hydroxyurea is the drug recommended upfront [7,34]. It has to be underlined that such recommendations are not strictly evidence-based, since no controlled randomized study comparing hydroxyurea to phlebotomy in high-risk patients with PV has been performed. Use of hydroxyurea in PV was introduced by the Polycythemia Vera Study group (PVSG) that, in a small randomized study (PVSG-01) comparing hydroxyurea to phlebotomy to radioactive phosphorus, concluded for an advantage for hydroxyurea as regarded reduction of cardiovascular events, although there was no significant benefit in overall survival [67]. In a subsequent small observation study, the PVSG-08, 51 PV patients treated with hydroxyurea experienced less thrombosis compared to expectations based on a historical control group [68]. However, an advantage of hydroxyurea is further supported by analysis of ECLAP data, where the rate of fatal/non-fatal cardiovascular events was reduced from 13.2% in patients treated with phlebotomy only to 7.9% in those receiving hydroxyurea [69]; such protective effect resulted greater for arterial than venous events, that indeed occurred at similar rate in the two groups of treatment [70]. An endless debate regarding leukemogenic potential of hydroxyurea is one major concern for the use of this drug, and is the ground on which to advocate phlebotomy only for managing the raised cell mass in any PV patients, irrespective of the risk category [9]; while for other chemotherapeutics, including ^{32}P, chlorambucil [71] and pipobroman [72], a clear increase of leukemic transformations was highlighted, long-term follow-up studies and registry data did not convincingly demonstrate an increased rate of acute leukemia in PV patients receiving hydroxyurea [39,73]. Unfortunately, large prospective studies assessing this specific aspect are very difficult to perform, considering the rarity of PV itself and even more of the occurrence of leukemic transformation, that sums up to 5–8% usually 10 to 15 years from diagnosis. Hydroxyurea is conversely well known to associate with an increased rate of non-melanoma skin cancers; concerning other solid tumors, in a comparison of 700 patients treated with hydroxyurea versus 342 managed with phlebotomy only in the ECLAP trial [74], no increase of solid tumors could be demonstrated. However, a conservative approach using the non-leukemogenic interferon-α is suggested in younger patients.

Recently, a new formulation of interferon (ropeginterferon alfa2b) received marketing authorization as "orphan drug" from the European Medicine Agency (EMA) for the treatment of patients with PV without symptomatic splenomegaly. Ropeginterferon is a single isomer, long acting pegylated interferon that is administered at 2–3 weeks interval. The approval was largely based on 36 months updated results of a phase 3, non-inferiority study comparing ropeginterferon to hydroxyurea/best available therapy (PROUD PV) [75]. At that time, complete hematologic remission (CHR) was recorded in 70.5% of patients compared to 51.4% for control arm, and CHR plus symptom control occurred in 52.6% versus 37.8%. One notable finding of this long-term follow-up was that hematologic, clinical and molecular responses increased progressively with ropeginterferon opposite to control arm. Molecular response was observed in 66.0% of ropeginterferon-treated patients compared to 27.0% of control arm, with a mean VAF change of −45% versus 5%; of note, reduction of $JAK2$V617F variant allele frequency correlated with CHR, in line with previous correlative studies of VAF and disease phenotype [76–78]. The rate of adverse events was comparable to control arm, and no unique new safety signal occurred. This new formation of interferon may have advantage in terms of efficacy and tolerability when compared to conventional and pegylated forms of interferon, that proved able to induce hematologic response, improvement of clinical manifestations, and varying reduction of the $JAK2$V617F mutated clone at variable extent in several small phase 2 studies [79]. A long term follow-up report with pegylated interferon alfa-2a confirmed sustained hematologic (median, 65 months) and molecular (median, 58 months) responses in 79% and 63% of PV patients, although major thromboembolic events and progression to myelofibrosis and leukemia were observed at a rate not far from what expected from historical matched cohorts of patients not treated with interferon [80]. In a randomized study of pegylated interferon (pegasys) versus hydroxyurea in 72 patients with high risk PV and ET, no major benefit of interferon for hematologic control, $JAK2$V617F allele burden reduction and BM morphology changes was observed, while tolerability was inferior [81]. Interferon is the drug of choice for the management of pregnancy in patients with PV and ET in cases where pregnancy is considered at "high-risk" for past history of pregnancy complications, previous thrombosis, or uncontrolled blood cell counts and/or symptoms [6,7,10,82].

Patients developing resistance or intolerance to hydroxyurea, as defined by the ELN criteria (Table 3), are candidate for second-line therapy [5]. Intolerance usually manifests with mucosal and cutaneous lesions, including oral, genital and leg ulcers; older subjects frequently develop actinic keratosis and non-melanoma skin cancers, and avoidance of sun exposure must be recommended. Hydroxyurea-related fever and interstitial pneumonitis are rare complications leading to withdrawal of the treatment. Development of cytopenias under hydroxyurea has been associated with risk of transformation to MF and AL, and an overall adverse outcome, in a retrospective study [83].

A part for intolerance, candidates for second line therapy are those patients who, in spite of adequate hydroxyurea dose, continue to present severe disease manifestations, including devastating pruritus, progressive and symptomatic spleen enlargement, or require a sustained, high rate of phlebotomy that results subjectively poorly tolerated. According to the ELN/IWG-MRT response criteria [62], definition of "responsive disease" should entail complete avoidance of phlebotomies; therefore, patients who require occasional phlebotomies under an optimally tolerated dose of hydroxyurea should be considered "resistant" to the drug, and eventually shifted to another treatment. However, there is no evidence that maintaining some phlebotomy requirement under hydroxyurea is predictive of increased risk of thrombosis [84]; according to our views, there is no reason to modify the ongoing treatment in these subjects just because they did not reach absolute phlebotomy independence.

Conventional drugs used for second-line treatment are interferon, for patients on hydroxyurea, and *viceversa*, or busulfan, that owing to its alkylating mode of action is reserved to older subjects [85]. The JAK1 and JAK2 inhibitor ruxolitinib was approved for PV patients with refractoriness or intolerance to hydroxyurea, based on results of two randomized studies, RESPONSE [86] and RESPONSE 2 [87]. Ruxolitinib was superior to BAT (that included also hydroxyurea) for maintenance of a hematocrit level < 45% without phlebotomies [88] and induced complete hematologic responses in 24% of the patients at primary endpoint assessment (32 weeks), as well as sustained reduction of spleen volume in 40% of the patients enrolled in RESPONSE study. The efficacy of ruxolitinib on hematocrit control was confirmed in the RESPONSE-2 study, performed in patients similarly resistant or refractory to hydroxyurea but without splenomegaly [87]; complete hematological remission was obtained in 24% of the patients on the ruxolitinib arm versus 3% in control arm at 80 weeks of follow-up [89]. Treatment was very effective in improving the symptomatic burden of the patients [90] and ameliorating quality of life [90]. Most patients have rapid, impressive improvements of their intractable pruritus and other burdening symptoms [91], that in the long-term might also benefit of the improvement of iron deficiency [65]. It is worth of mention, however, that in a double-blind, double-dummy phase 3 b study, RELIEF study, aimed at evaluating the impact of ruxolitinib on symptomatic improvements in patients reporting PV-related symptoms on a stable dose of hydroxyurea, only a nonsignificant trend towards symptomatic improvement with ruxolitinib was observed [92]. At 80-week follow-up, a reduction in the number of thromboembolic events was observed in the ruxolitinib arm (1.8 per 100 patient-years) compared to BAT (8.2 per 100 patient-years) on the RESPONSE trial [88]. A meta-analysis of JAK2V617F VAF changes up to weeks 208 in ruxolitinib-randomized patients disclosed mean changes from baseline VAF ranging from −12.2 to −40.0%, whilst complete or partial molecular response occurred in a minority, suggesting that treatment with ruxolitinib for as long as 4 years may induce some degree of reduction in $JAK2$V617F VAF but major/complete responses are very infrequent; furthermore, although the relationships of reduced VAF with clinical

Table 3

Definition of resistance and intolerance to hydroxycarbamide (HU) in patients with PV and ET according to the ELN consensus.

Polycythemia Vera	Essential Thrombocythemia
• Need for phlebotomy to keep hematocrit < 45% after 3 months of at least 2 g/day of HU, OR • Uncontrolled myeloproliferation, i.e. platelet count > 400 × 10⁹/L AND white blood cell count > 10 × 10⁹/L after 3 months of at least 2 g/day of HU, OR • Failure to reduce massive splenomegaly (i.e., extending > 10 cm from the left costal margin) by more than 50% as measured by palpation, OR failure to completely relieve symptoms related to splenomegaly, after 3 months of at least 2 g/day of HU, OR • Absolute neutrophil count < 1.0 × 10⁹/L OR platelet count < 100 × 10⁹/L or hemoglobin < 100 g/L at the lowest dose of HU required to achieve a complete or partial clinico-hematological response*, OR • Presence of leg ulcers or other unacceptable HU-related non-hematological toxicities, such as mucocutaneous manifestations, gastrointestinal symptoms, pneumonitis or fever at any dose of hydroxycarbamide	• Platelet count > 600 × 10⁹/L after 3 months of at least 2 g/day of HU (2.5 g/day in patients with a body weight > 80 kg). • Platelet count > < 400 × 10⁹/L and leukocyte count < 2.5 × 10⁹/L at any dose of HU. • Platelet count > < 400 × 10⁹/L and hemoglobin less than 10 g/dL at any dose of HU. • Presence of leg ulcers or other unacceptable mucocutaneous manifestations, at any dose of HU. • HU-related fever.

outcomes remained unclear [93]. Analysis of patients treated with interferon in the RESPONSE trial also disclosed superiority of ruxolitinib to interferon in the randomized treatment arms, as well as after cross-over from previous interferon to ruxolitinib [94].

6. Treatment of patients with ET

Current indications for the treatment of patients with ET are largely based on the results of some randomized studies. In a trial conducted in 1995 ("Bergamo trial"), 114 patients at high risk of thrombosis were randomly assigned to receive hydroxyurea or no myelosuppressive therapy, on top of a antiplatelet agent (aspirin or ticlopidine); the rate of thrombosis resulted significantly reduced from 24% to 3.6% at 27 months in patients receiving hydroxyurea, providing direct evidence of a protective effect of cytoreduction in high-risk patients [95]. Hydroxyurea resulted superior also to anagrelide in reducing arterial thrombosis, major bleeding and progression to myelofibrosis in the Primary Thrombocythemia-1 (PT-1) study, that randomized 809 patients at high risk for vascular events who were concurrently receiving low-dose aspirin [96]; after a median follow-up of 39 months, the odds ratio for all thrombosis resulted of 1.57 (95% confidence interval, 1.04 to 2.37) in the anagrelide group compared with hydroxyurea. Conversely, anagrelide performed better for prevention of venous events (odds ratio, 0.27, 95%CI 0.11 to 0.71). Of note, control of platelet count was similarly effective in the two study arms. Anagrelide was also associated with higher likelihood of transformation to myelofibrosis, with an estimated actuarial risk at 5 years of 2% for hydroxyurea versus 7% for anagrelide. A subsequent study, the ANAHYDRET study, completed in 2013, included 259 previously untreated high-risk patients with ET whose diagnosis had been performed according to the WHO criteria, thereby excluding patients with ET-mimicking prePMF that had been enrolled in the PT-1 study; patients were randomized to receive anagrelide or hydroxyurea. Results indicated that in patients with WHO-defined ET, anagrelide did not prove inferior to hydroxyurea in reducing arterial and venous thrombosis, and there was no significant increase of bleeding [97]. Finally, in the randomized, double-blind active controlled, TEAM-ET trial, a anagrelide prolonged-release formulation was compared to reference product in 106 patients; the study showed non-inferiority of new formulation over the reference in reducing platelet count. Unfortunately, vascular events were not an endpoint of the study [98]. Combination of hydroxyurea and anagrelide in patients whose platelet counts was poorly controlled with monotherapy were shown safe and effective in an observational study in 347 patients [99].

The safety and efficacy of aspirin as primary prophylaxis in patients with ET is not based on prospective controlled studies. In low-risk *CALR* mutated patients receiving antiplatelet agents, no advantage on thrombosis rate and an excess of hemorrhage was shown in a large retrospective analysis [100], while some benefit was seen in patients with *JAK2*V617F mutation or generic cardiovascular risk factors [101]. On the other hand, preclinical evidence would support increasing benefit of low-dose aspirin twice a day, due to an accelerated renewal of platelet cyclooxygenase-1 owing to increased production of platelets from megakaryocytes [102,103]. A prospective trial (ARES) is ongoing to experimentally test this hypothesis [104]. Low-dose aspirin is usually quoted effective in mitigating microvascular symptoms, whereas higher dose may be required for painful erythromelalgia or severe headache attacks.

The question whether thrombocytosis should be cytoreduced in any patient, especially those presenting with "extreme thrombocytosis" independent of conventional risk categories, is still debated. However, since there is no evidence of thrombocytosis being correlated with thrombosis, normalization of platelet count should not be considered a goal of therapy, according to most experts; therefore, it is not recommended to start cytoreduction in otherwise asymptomatic low-risk patients with platelet count up to $1500 \times 10^9/L$. However, if thrombocytosis exceeds 2 million, and in any case when the subject is referring microvascular symptoms or presents hemorrhagic manifestations, it is prudent to institute cytoreductive therapy. When platelets are above one/one and half million, it is also prudent to stop aspirin owing to a greater risk of bleeding; in cases where anti-platelet agents are desirable due to cardiovascular comorbidities or because of microvascular disturbances, measurement of vonWillebrand factor' levels and activity is recommended to support decision to continue aspirin. In selected cases where rapid reduction of extreme thrombocytosis is required, plateletpheresis is an option.

Management of specific situations: splanchnic vein thrombosis. The most common, challenging "specific situations" that may be encountered in patients with PV and ET, at diagnosis or during the course, are represented by the need of treating an acute thrombotic event or major hemorrhagic complications, or managing a pregnancy; guidelines and consensus indications have been incorporated, among the others, in the ELN, NCCN and the British Society of Hematology, to which the reader is referred [6,105,106].

A relatively frequent situation that may occur, particularly as the event leading to a diagnosis of PV or ET, but also during follow-up of an established disease, is represented by thrombosis in "unusual" sites, that include the splanchnic vein district, cerebral vein thrombosis and central retinal vein [107]. Splanchnic vein thrombosis (SVT) include Budd-Chiari syndrome, portal vein thrombosis, splenic and mesenteric vein thrombosis [108–110]. While SVT are very rare events in the general population (0.7 to 2.7/100,000 person-years) [111], MPN represent a common cause of SVT accounting for up to 10% of all SVT and 50–60% of Budd-Chiari syndrome. Therefore, screening for *JAK2*V617F mutation (and in selected *JAK2* unmutated cases, for *MPL* and *CALR* mutation, although very rarely occurring) is recommended in the work-up of any patients presenting with SVT, since this allows to identify 15–17% of cases that lack the traditional hallmarks of MPN and would otherwise be missed [112]. In fact, in cases where the SVT is the index event eventually leading to MPN diagnosis, recognition of an underlying MPN may be challenging owing to the fact that peripheral blood cell counts are often minimally abnormal. Although results of prospective, controlled studies are not available, there is a general experts' agreement [6,113] in maintaining a long-term anticoagulation in these patients due to the high rate of recurrence, either in the splanchnic vein district itself, other venous vessels and, yet at a lower rate, in arterial vessels [114,115]. On the other hand, the impact of cytoreduction, usually recommended for the high-risk characteristics of these patients who experienced such thrombotic events, was questioned more recently in a study where no impact of hydroxyurea in preventing recurrence was shown [115]. While there is yet no obvious explanation for this finding, one might speculate that, owing that most patients with SVT have normal, or borderline increased, levels of blood cells, the role of cytoreduction is less crucial than in other situations The use of the JAK1 and JAK2 inhibitor ruxolitinib has been shown to be safe and effective in patients with SVT associated with PV, ET and also PMF [116]; preliminary data with interferon alfa indicate safety and efficacy (Mascarenhas J, submitted). The pathogenesis of MPN-associated SVT is debated; of interest, endothelial cells harboring the *JAK2*V617F mutation have been demonstrated in liver endothelial cells isolated by microdissection in patients with MPN-associated Budd-Chiari [117] as well as in circulating endothelial progenitor cells [118–120].

7. What is needed to move the field forward

There are several aspects in this field that, once clarified, might indeed represent significant steps forward for adequate and successful management of PV and ET. Some of the most compelling in authors' view are listed below.

● Clarifying the nature of "triple negative" patients with ET: are these true myeloproliferative neoplasms, which is their course, if and how they should be treated.

- Improving the thrombosis risk stratification of patients with PV: can mutational genetics help in better delineating categories of patients in need of treatment?
- Understanding the long-term significance of interferon-induced molecular remissions in PV: how stable are they, what is their ultimate impact on thrombosis, transformation to MF and acute leukemia, and survival.
- Delineating benefits and risks of novel drugs for first- and second-line therapy in PV and ET. Where should be the border for shifting from one drug to the other? Which is the final goal: better disease control and prevention of progression, cure? What the safety in the very long-term? And what about sustainability of costs?

Practice points

- We strictly adhere to the 2016 WHO criteria for establishing diagnosis of PV and ET, and the IWG-MRT criteria for diagnosis of PPV-MF and PET-MF.
- We perform (in the order) *JAK2*V617F and *JAK2*exon 12 mutation search in all patients with suspected PV, and *JAK2*V617F, *CALR* and *MPL*W515 mutation (in the order) for those with thrombocytosis and suspicion of ET.
- We do not routinely assess non-driver myeloid mutations, except to establish clonal hematopoiesis in triple-negative cases of thrombocytosis with a BM biopsy suggestive of ET or patients with JAK2 unmutated erythrocytosis.
- We do not perform serial BM biopsies unless in the suspicion of evolution to PPV- and PET-MF or acute leukemia, but we do BM biopsy in any suspected case of ET and PV at diagnosis (with some exceptions, as noted above, or very old subjects).
- We use thrombosis risk score for PV and revised IPSET score for ET to tailor therapy.
- We adopt ELN recommendations for treatment.

Research agenda

- Definition of mechanisms of response, including molecular response, to ruxolitinib and interferon, and other new drugs as well
- Identification of molecular predictors of major clinical events, including thrombosis and hematological progression
- Clinical trials for new antithrombotic agents, including new inhibitors of platelet function and direct anticoagulants

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