From leeches to personalized medicine: evolving concepts in the management of polycythemia vera

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ABSTRACT

Polycythemia vera is a clonal disorder of hematopoietic stem/progenitor cells. It manifests as an expansion of red cell mass. It is the most common chronic myeloproliferative neoplasm. In virtually all cases, it is characterized by a V617F point mutation in *JAK2* exon 14 or less common mutations in exon 12. The landmark discovery of the autonomously activated JAK/STAT signaling pathway paved the way for the clinical development of the first target drug, the JAK1 and JAK2 inhibitor ruxolitinib. This is now approved for patients with resistance or intolerance to hydroxyurea. Phlebotomies and hydroxyurea are still the cornerstone of treatment, and aim to prevent the first appearance or recurrence of cardiovascular events that, together with progression to post-polycythemia vera myelofibrosis and leukemia, represent the main causes of death. Interferon-α is an alternative drug and has been shown to induce molecular remissions. It is currently undergoing phase III trials that might eventually lead to its approval for clinical use. The last few years have witnessed important advances towards an accurate early diagnosis of polycythemia vera, greater understanding of its pathogenesis, and improved patient management. This review will focus on the most recent achievements and will aim to unify the different concepts involved in a personalized approach to the patient with polycythemia vera. In spite of many recent advances in the understanding of its pathogenesis and improved disease management, polycythemia vera remains a life-threatening myeloproliferative neoplasm for which there is no cure. This review will present a critical overview of evolving concepts in diagnosis and treatment of this disease.

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Introduction

If I ask my daughter to give me three key dates in the history of Rome, I expect her to say: 753 BC (the foundation of the city), 44 BC (the murder of Julius Cesar) and 476 AD (the fall of the Roman Empire). If I am asked to provide three key dates for polycythemia vera (PV), I will say: 1892, the date of the first description by Louis Henri Vaquez which was then reinforced by William Osler's report in 1903,[1] 1951, when William Dameshek grouped together PV, myelofibrosis (MF) and essential thrombocythemia (ET) under the term "myeloproliferative disorders",[2] and 2005, when William Vainchenker,[3] Tony Green,[4] Ross Levine[5] and Robert Kralovics[6] independently described the *JAK2*V617F mutation. It is fascinating that the original speculation by Vaquez that PV was due to hematopoietic hyperactivity, and the illuminating hypothesis of Dameshek that all myeloproliferative disorders [now known as myeloproliferative neoplasms (MPN)][7] variably reflected increased proliferative activity of bone marrow (BM) cells "due to a hitherto undiscovered stimulus",[2] were both reconciled by the demonstration of abnormal activation of JAK/STAT signaling as the unifying pathogenetic mechanism. However, beyond these groundbreaking discoveries, the history of PV is punctuated by achievements that have contributed to various degrees to improve our understand-

ing to the level of knowledge we have now. Table 1 lists some of these landmark studies; due to space constraints, I will not be able to address all of them in detail.

Evolving concepts in diagnosis

Making a diagnosis of polycythemia vera

The World Health Organization (WHO) recently released a revised classification of MPN in which important changes to the 2008 version were introduced (Table 2).[8] In the 2008 version, the most compelling innovation had been the introduction of *JAK2*V617F and "similar" mutations (involving *JAK2* exon 12 in 3%-4% of patients) as major diagnostic criteria.[3-6] Although *JAK2*V617F mutation is associated with PV in more than 95% of cases, it does not represent a clear diagnosis since it is found also in 50%-60% of ET and PMF. However, the use of *JAK2*V617F as a marker of clonal myeloproliferation greatly facilitates the distinction of PV from reactive or congenital erythrocytosis.

Considering that isotope-based assays for measuring red cell mass (RCM) and plasma volume are not routinely available even in most tertiary centers, the 2008 WHO classification listed a hemoglobin level more than 185 g/L and 165 g/L in men and women, respectively, as a strong surrogate marker of absolute increase of RCM. Since some PV patients do not fulfill such high levels, other criteria were added to facilitate diagnosis, including: 1) hemoglobin or hematocrit level that is more than 99th percentile of reference range for age, sex, or altitude of residence; 2) an RCM that is more than 25% above mean normal predicted value; 3) a hemoglobin level more than 170 g/L and 150 g/L in men and women, associated with a sustained increase of 20 g/L from baseline not attributable to correction of iron deficiency. According to the pragmatic British standards, hematocrit more than 52% in males and more than 48% in females, or an RCM more than 25% above predicted value, are sufficient to establish a diagnosis of PV if *JAK2* mutation is present.[9] However, a reassessment of how far the WHO criteria can be applied in a real-life setting raised the issue of *JAK2*V617F mutated patients with only a borderline increase in hemoglobin. It was shown that BM morphology, according to WHO guidelines, accurately reflected a condition of increased RCM, since all patients with increased RCM also had a BM morphology consistent with PV.[10] In 140 such patients, Barbui *et al.* delineated a category operationally defined as "masked" PV[11] that includes a majority of early cases, in which thrombocytosis is the initial disease manifestation, mimicking ET. Additional features that distinguish masked from overt PV include male predominance, higher incidence of arterial thrombosis and progression to post-PV myelofibrosis (PPV-MF) and acute leukemia (AL), resulting in inferior survival. Therefore, masked PV is a heterogeneous condition including early forms of PV as well as a distinct phenotype with a more aggressive course. The identification of masked PV might also reconcile differences in reported incidence of transformation of *JAK2*V617F mutated ET to PV.[12-14] The best cut off for hemoglobin/hematocrit to discriminate *JAK2*V617F mutated ET from PV was set at 165 g/L/49% in males and 160 g/L/48% in females.[15]

These findings constituted the backbone for the 2016

Table 1. Landmark studies in understanding polycythemia vera and its diagnosis and management.

Domain	Field of investigation/Study	Findings / Comments
Natural history		
	• Description	• First description by L. Vaquez, first review by W. Osler[1]
	• Classification	• W. Dameshek theorizes the concept of "myeloproliferative disorders"[2]
	• Natural history	• A cohort study on the natural history of PV by the Gruppo Italiano Studio Policitemia[111]
	• Natural history	• An international study on natural history of contemporary PV patients[23]
Pathophysiology		
	• Clonality	• Involvement of a hematopoietic stem/progenitor cell established by analysis of G6PDH isoenzymes[112]
	• EEC	• Cytokine independent growth of erythroid progenitor cells[16]
	• Molecular basis	• Description of the *JAK2*V617F mutation[3-6]
	• Molecular basis	• Description of mutations in JAK2 exon 12[113]
	• Molecular basis	• Occurrence of non-driver somatic mutations[114]
Diagnosis		
	• PVSG criteria	• Development of formal diagnostic criteria[40]
	• WHO 2008	• Introduces *JAK2* mutations as major diagnostic criteria[7]
	• WHO 2016	• Introduces BM biopsy as major diagnostic criterion and adopts the concept of "masked" PV[8]
Management		
	• Thrombosis and hematocrit	• Points to hematocrit >45% as main risk factor for thrombosis[77]
	• PVSG-01 trial	• Use of phlebotomy; leukemogenic risk of ^{32}P and chlorambucil[39]
	• PVSG-08 trial	• Efficacy of hydroxyurea[115]
	• ECLAP trial	• Low-dose aspirin for prevention of CV events[55]
	• CytoPV trial	• Evidence-based setting of the optimal hematocrit level at <45%[79]
	• FPSG long-term trial	• Leukemogenic risk with pipobroman[41]
	• Interferon study	• First sound evidence of an impact of interferon on molecular remission[86]
	• RESPONSE trial	• Ruxolitinib for patients with resistance/intollerance to hydroxyurea[73]

G6PDH: glucose-6-phosphate dehydrogenase, an X-linked locus; EEC: endogenous erythroid colonies; CV: cardiovascular; PVSG: Polycythemia Vera Study Group; Cyto-PV: Cytoreductive Therapy in PV trial; ECLAP: European Collaboration on Low-dose Aspirin in PV trial; RESPONSE: Randomized Study of Efficacy and Safety in Polycythemia Vera with JAK Inhibitor INCB018424 versus Best Supportive Care; FPSG: French Polycythemia Study Group.

revision of WHO criteria, where main changes regarded the threshold level of hemoglobin/hematocrit, the upgrade of BM biopsy to major criterion, and the abandonment of endogenous erythroid colony assay[16] as minor criterion[8] (Table 2). Subnormal erythropoietin levels remain the only accessory criterion, although in more than 20% of cases the levels fall within normal range. It has been argued that these novel criteria might promote an increased usage of BM biopsy in the diagnostic path of erythrocytosis. However, in *JAK2* mutated cases that present hemoglobin levels fulfilling the 2008 criteria, biopsy is not required for diagnosis, although it may be recommended, especially in younger subjects, to assess initial fibrosis that predicts an accelerated progression to PPV-MF.[17] Conversely, biopsy is mandatory when hemoglobin/hematocrit are at the lower threshold level set by the 2016 criteria, and early PV must be distinguished from *JAK2*V617F mutated ET. Misdiagnosis with ET would mean that many patients would only receive suboptimal control of hematocrit.[18]

Diagnosis of transformation to post-polycythemia vera myelofibrosis

Post-polycythemia vera myelofibrosis (PPV-MF) represents a natural evolution of PV. Diagnostic criteria have been outlined by the International Working Group-Myeloproliferative neoplasms Research and Treatment (IWG-MRT) expert consensus (Table 3).[19] The major criterion is the development of BM fibrosis grade 2 or higher (in the European scale;[20] ≥grade 3 in the conventional scale[21]) in the context of a previous diagnosis of PV. It is worthy of note that the 2016 WHO revision enlists criteria for semiquantitative grading of BM fibrosis on a scale from 0 to 3. Additional variables, two of which are required to establish diagnosis, are: 1) anemia or sustained loss of need for phlebotomy and/or cytoreductive therapy; 2) leukoerythroblastic peripheral blood; 3) the new appearance, or progression, of splenomegaly; 4) development of constitutional symptom(s).

Based on several small historical series (reviewed by Cerquozzi and Tefferi[22]) and a recent large study with mature survival data,[23] the median time to myelofibrosis transformation ranges from 8.5 to 20 years and the cumulating risk increases from 6% to 14% to 26% at 10, 15 and 20 years, after the initial diagnosis, respectively. Older age, leukocytosis, high *JAK2*V617F allele burden (that usually increases further along with transformation),[24,25] splenomegaly and thrombocytosis have all been associated with increased risk of PPV-MF.[23-26] More recently, the independent value of BM fibrosis at diagnosis of PV[17] and the clinical phenotype of masked PV were recognized. Of 526 PV patients, 14% showed grade 1 fibrosis; this group was characterized by a higher prevalence of palpable splenomegaly and greater risk of progression to overt myelofibrosis [incidence rate (IR) 2.2 per 100 patient-years *vs.* 0.8 for those without fibrosis].[17] Furthermore, the combined rate of transformation to PPV-MF and AL was significantly higher among patients with masked PV compared with overt PV (1.60 *vs.* 0.97 *per* 100 patient-years, respectively). Preliminary evidence suggests that chromosome 12 abnormalities are associated with a greater likelihood to progress to PPV-MF.[27]

Occurrence of PPV-MF signifies a dramatic shortening of PV survival to a median of approximately six years with an adjusted hazard ratio (HR) of 2.17.[26] A longer (>10 years) duration of the chronic PV phase is also associated with shortened survival after transformation to PPV-MF (HR 2.26).[28] According to a dynamic prognostic model, presence of any of 3 independent variables (anemia, thrombocytopenia and leukocytosis) resulted in a 4.2-fold increase in the risk of death; in particular, occurrence of anemia at PPV-MF was associated with shortened survival (1.9 *vs.* 6.6 years for non-anemic patients).[26] However, in clinical practice, and in clinical trials,[29,30] prognostication assessment of PPV-MF patients is usually performed with the International Prognostic Scoring System (IPSS) and the dynamic International Prognostic Scoring System (DIPSS), originally developed for PMF.[31,32] In fact, these scores have not been

Table 2. The 2016 WHO revised diagnostic criteria for polycythemia vera.[8]

Major criteria:
1. Hemoglobin > 165 g/L or, Hematocrit > 49% in men Hemoglobin > 160 g/L or, Hematocrit > 48% in women or, increased red cell mass*
2. BM biopsy 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
Minor criterion: Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all three major criteria, or the first two major criteria and the minor criterion**

WHO: World Health Organization; BM: bone marrow; PV: polycythemia vera. *More than 25% above mean normal predicted value. **Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels more than 185 g/L in men (hematocrit 55.5%) or more than 165 g/L in women (hematocrit 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

Table 3. The IWG-MRT recommended diagnostic criteria for post-polycythemia vera myelofibrosis.[19]

Required criteria:
1. Documentation of a previous diagnosis of PV as defined by the WHO criteria
2. BM fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale)*
Additional criteria (two are required):
1. Anemia** or sustained loss of requirement for 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 LCM) or the appearance of a newly palpable splenomegaly
4. Development of ≥ 1 of 3 constitutional symptoms: > 10% weight loss in 6 months, night sweats, and unexplained fever (> 37.5°C)

IWG-MRT: International Working Group for Myeloproliferative neoplasms Research and Treatment; PV: polycythemia vera; WHO: World Health Organization; BM: bone marrow; LCM: left costal margin. Diagnosis is made with the 2 required criteria plus 2 additional criteria. *Grade 2–3 according to the European classification[20]: diffuse, often coarse fiber network with no evidence of collagenization (negative trichrome stain) or diffuse, coarse fiber network with areas of collagenization (positive trichrome stain). Grade 3–4 according to the standard classification[21]: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis or diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis. **Below the reference range for appropriate age, sex, and altitude considerations.

validated in PPV-MF and they may not perform adequately in distinguishing different risk categories.[28,33] Finally, although the mutation landscape of PPV-MF has similarities with PMF,[34] in contrast with PMF, little impact of mutations on prognosis was demonstrated.[28]

Diagnosis of transformation to blast phase

A consensus has been achieved as to the definition of accelerated and blast phase disease in PV (and other MPN) as being characterized by peripheral or BM blast percentages of 11%-19% and more than 20%, respectively.[35] Rate of transformation to AL is estimated at 2%, 5%, and more than 10% at 10, 15 and 20 years.[23,36] Risk factors for leukemic transformation include advanced age, leukocytosis, splenomegaly and abnormal karyotype.[22,23] There is no specific molecular marker that is predictive of blast transformation; interestingly, leukemic blasts may result *JAK2* wild type, suggesting the emergence of an unrelated leukemic clone.[37]

The promoting role of cytotoxic therapy in the events leading to blast transformation of PV remains a subject of major debate.[38] The leukemogenic potential of ^{32}P and alkylating agents (chlorambucil and pipobroman) was demonstrated by the PVSG[39,40] and the French Polycythemia Study Group.[41] The randomized PVSG-01 study reported an excess of late-appearing AL in patients treated either with chlorambucil or ^{32}P (13.2% and 9.6%, respectively) compared with phlebotomy (1.5%).[39] The latest update after a median follow up of 16 years of a French study that randomized PV patients under 65 years of age to receive pipobroman or hydroxyurea as first-line therapy reported significantly shorter survival in the pipobroman group (15.4 years *vs.* 20.3 years for patients treated with hydroxyurea) and significantly higher cumulative incidence of leukemia (13%, 34% and 52% *vs.* 6.6%, 16.5%, 24% for hydroxyurea, at 10, 15 and 20 years), although transformation to PPV-MF was lower in the pipobroman group (21% *vs.* 32% at 20 years).[41] Similar findings were reported in a retrospective cohort of more than 1500 patients with PV;[23] in this study, the use of hydroxyurea or busulphan alone was not burdened with increased leukemia rate, similar to findings of the prospective ECLAP cohort.[42] However, the use of 2 or more cytotoxic agents, including hydroxyurea, was associated with a 2.9 increased odds of leukemia.[43] Although it is not possible to verify whether such an increased rate of transformation in patients receiving multiple lines of thera-

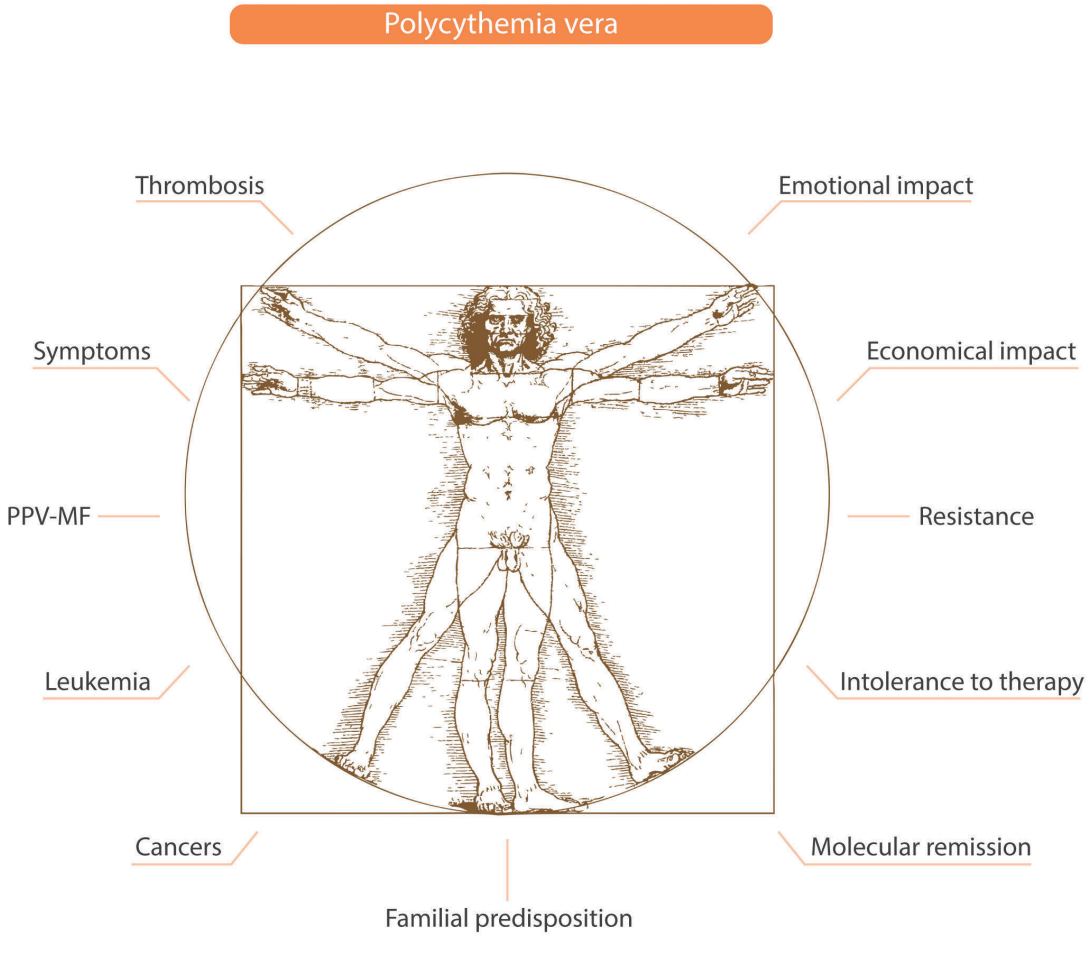

Figure 1. The burden of disease in a patient with polycythemia vera. Shown is the famous drawing Uomo Vitruviano of Leonardo da Vinci (1490), named after the ancient roman architect Vitruvius. Here the ideal man is represented as perfectly inscribed in both a square and a circle. In the figure, this concept is used to signify the appropriateness of a modern approach to the patient with PV that ideally takes into account the multiplicity of aspects associated with the disease.

py is directly caused by drugs or rather reflects a more aggressive disease, the decision to shift to second-line therapy in patients previously treated with hydroxyurea must consider the risk associated with the use of multiple cytotoxic agents. In this regard, there is no evidence of a leukemogenic effect of interferon.[44] Furthermore, in a nested-case control study of 162 MPN patients, of which the majority (68%) were PV, 25% of those who transformed to AL had never been exposed to cytotoxic therapy, thereby reinforcing the contention that individual genetic characteristics are themselves causative of the inherent propensity of PV to transform to AL or myelodysplastic syndrome (MDS).[43]

Evolving concepts in understanding predisposition to polycythemia vera

Polycythemia vera, as all MPN, shows a familial aggregation whereby it has been calculated that first-degree relatives have a 5-7-fold higher risk of developing an MPN in comparison to the general population.[45,46] Clinical presentation, rate of thrombosis and survival of familial cases are similar to sporadic MPN.[46,47] The *JAK2*V617F mutation is acquired somatically in familial cases of PV as in sporadic patients. The genetic basis of familial aggregation of MPN have not yet been clarified, although it is likely that patients inherit some predisposition to acquire one of the driver mutations.[46] In sporadic cases, the *JAK2* 46/1 haplotype has been associated with the acquisition of *JAK2*V617F mutation.[48,49] A high incidence of PV among Ashkenazi Jewish descent has been described,[50] but there are no clues as to genetic background.

No association between an excess risk of PV and blood donation or donation frequency has been observed in a study involving 1.4 million donors,[51] refuting previous reports in smaller series.[52]

Evolving concepts in patient management

Risk stratification

Polycythemia vera is associated with reduced life expectancy, primarily because of hematologic progression and cardiovascular events.[23,36,53] Analysis of the most mature survival data clearly shows the shorter life expectancy. Among 337 patients followed at the Mayo Clinic, of whom 44% died, median survival was 14.1 years; significantly shorter than the control population.[23] Risk factors for overall survival independent of the cause included advanced age, leukocytosis, venous thrombosis, and abnormal karyotype. Median survival was 10.9 and 27.8 years in high- and low-risk patients, respectively[23] (Table 4). However, this score is not used for decision making in clinical practice.

Approximately 15% of patients with PV may experience a thrombotic event during the disease course. Major thrombotic events are transient ischemic attacks, stroke, myocardial infarction, deep venous thrombosis or pulmonary embolism, peripheral arterial and venous thrombosis. Microvascular symptoms, such as hearing or visual impairments, paresthesia, or headache, are common. Venous thrombosis in unusual sites, particularly the splanchnic veins (SVT; portal, mesenteric, splenic), Budd-Chiari syndrome, thrombosis of the cerebral venous sinuses and central retinal vein are more frequent than in the general population. History of thrombosis is the main risk factor for recurrent cardiovascular events that occurred in the same vessel district as the first event in 75% and 61% of arterial and venous thrombosis, respectively;[54] history of hypertension predicts for arterial thrombosis and advanced age predicts for venous thrombosis. The frequency of arterial (16%) and venous (7.4%) thrombosis in 1818 patients diagnosed in the last decade was lower than in previous historical cohorts, including the ECLAP study (27% and 11%, respectively),[55,56] but was similar to the contemporary Cyto-PV study (arterial 17%, venous 12%) and the Swedish registry.[57] However, it is remarkable that while a reduction of thromboses from 4.01 to 2.93 *per* 100 patient-years was seen in the "high-risk" category, the rate of vascular events was unchanged in the "low-risk" category (2.03 *vs.* 2.24), thereby suggesting some under-treatment of these conventionally-defined low-risk subjects.[58] This is supported by the unexpected higher rate of thrombosis in young patients (age < 40 years) with masked PV compared with overt PV (3.01 *vs.* 1.99 *per* 100 patient-years, respectively). In multivariate analysis, the only factor accounting for such a difference was the less frequent use of phlebotomies and cytoreduction in younger patients with masked PV.[18]

The current risk stratification, informing therapeutic decisions, is designed to estimate the likelihood of developing thrombotic complications, and not necessarily the overall survival (Table 4). Age of 60 years or over and history of previous thrombosis are used to classify patients into a low- (neither present) or high- (either present) risk category. An important element for risk stratification is the comprehensive assessment of additional risk factors for thromboembolism, including smoking,[59] hypertension, diabetes, abnormal lipid levels, and obesity. The individual should be made aware of the value of a healthy life style in minimizing thrombotic risk, and encouraged to adopt appropriate

Table 4. Criteria used for risk stratification in polycythemia vera.

Criteria	Variables	Risk categories	Used for risk-adopted therapy
Thrombotic risk[56]	• Age > 60 • Previous thrombosis	• Low (neither present) • High (either present)	Yes
Shortened survival risk[23]	• Age (57-66 years = 2 points) (≥ 67 years = 5 points) • Leukocytes > $15x10^9$/L (= 1 point) • Venous thrombosis (= 1 point)	• Low (0 points) • Intermediate (1-2 points) • High (> 3 points)	No
Thrombosis	• Leukocytosis[59] • *JAK2*V617F allele burden[60] • Generic cardiovascular risk factors[59]		Not yet formally included in risk scores

measures to correct bad habits. However, generic cardiovascular risk factors, as well as leukocytosis[59] and higher *JAK2*V617F allele burden,[60] that have all been associated with higher risk of thrombosis, are not formally integrated into current scores.

Recognizing the burden associated with disease

Although symptoms typically associated with PV have been well known since initial descriptions, it is only recently that a full appreciation of their complexity, extent and impact has been acknowledged.[61] Components of the disease-associated burden include symptomatic manifestations (especially, but not limited to, fatigue, pruritus, symptoms due to splenomegaly, constitutional symptoms), reduced quality of life, the emotional impact, the financial impact of increased healthcare utilization and impaired incomes. Pruritus, typically acquagenic, is the most frequent and disabling complaint of patients with PV, and is reported in up to 70% of cases.[62] In extreme situations (approx. 15%)[63] it causes severe disruption of the individual's lifestyle, inducing sleep disturbances, depression, and impaired working capabilities and social relationships. In a recent landmark study into MPN in the United States that interviewed 380 PV patients undergoing treatment, fatigue and itching were identified by 33% and 9% of the respondents as the symptom they most urgently wanted to resolve.[64] The pathogenetic link between symptoms and clonal myeloproliferation is likely sustained by an abnormal release and signaling of inflammatory cytokines through the deranged JAK/STAT pathway,[65] a concept that is reinforced by the unique and rapid symptomatic efficacy of the JAK1 and JAK2 inhibitor ruxolitinib.[66]

To add to the burden associated with PV, one must consider the side effects of treatment, including worsening of fatigue and other signs of iron deficiency in heavily phlebotomized patients, varying manifestations of intolerance to hydroxyurea, the known toxicities of interferon, the increased Herpes Zoster reactivation with ruxolitinib, to name but a few. Presence of splenomegaly, use of hydroxyurea, and phlebotomy requirement are all independently associated with a substantial symptom burden.[67] Interestingly, a high symptomatic burden may occur independently of conventional risk categories; therefore, some low-risk patients might remain under-managed according to current recommendations.[67] Another component of the PV-associated burden is the high incidence of co-existing hematologic or solid cancers. In a study including 353 PV patients, a 3.44-fold increased risk of lymphoproliferative neoplasms, especially chronic lymphocytic leukemia, compared with the general population, was reported.[68] Among 2000 MPN patients from cancer registries, the prevalence of all types of cancer was higher than in the general population; in PV patients there was a significantly higher risk of malignant skin melanoma.[69]

It is remarkable that recognition of disease-associated burden, and the development of standardized approaches for its quantification,[70-72] such as the Myeloproliferative Neoplasm Symptomatic Assessment Form (MPN-SAF),[70] have been fostered by the development of JAK2 inhibitors that proved unforeseen efficacy to ameliorate symptomatic manifestations of MPN.[29,30,66] It is worthy of note that such scores have been integrated into the pivotal study leading to approval of the use of ruxolitinib in PV.[73]

Defining end points for treatment

According to the European Leukemia Net (ELN) consensus criteria, the goals of therapy in patients with PV are to reduce the risk of first and/or recurrent thrombosis, prevent bleeding events, minimize the risk of evolution to PPV-MF and AML, and ameliorate the symptom burden.[74] Revised response criteria were released recently by the ELN and IWG-MRT[75] (Table 5). Three levels of responses are enlist-

Table 5. Response criteria for polycythemia vera according to the ELN and IWG-MRT consensus.[75]

Complete remission	
A	Durable* resolution of disease-related signs including palpable hepatosplenomegaly, large symptoms improvement† AND
B	Durable* peripheral blood count remission, defined as Ht lower than 45% without phlebotomies; platelet count < 400x10⁹/L, WBC count < 10x10⁹/L, AND
C	Without progressive disease, and absence of any hemorrhagic or thrombotic event, AND
D	Bone marrow histological remission defined as the presence of age-adjusted normocellularity and disappearance of trilinear hyperplasia, and absence of > grade 1 reticulin fibrosis
Partial remission	
A	Durable* resolution of disease-related signs including palpable hepatosplenomegaly, large symptoms improvement† AND
B	Durable* peripheral blood count remission, defined as Ht lower than 45% without phlebotomies; platelet count < 400x10⁹/L, WBC count < 10x10⁹/L, AND
C	Without progressive disease, and absence of any hemorrhagic or thrombotic event, AND
D	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

ELN: European LeukemiaNet; IWG-MRT: International Working Group-Myeloproliferative neoplasms Research and Treatment; Ht: hematocrit; WBC: white blood cell count; PV: polycythemia vera. *Lasting at least 12 weeks. †Large improvement in symptom(s) (≥10-point decrease) in MPN-SAF TSS.[10] Molecular response is not required for assignment as complete response or partial response. Molecular response evaluation requires analysis in peripheral blood granulocytes. Complete response is defined as eradication of a pre-existing abnormality. Partial response applies only to patients with at least 20% mutant allele burden at baseline. Partial response is defined as 50% or more decrease in allele burden.

ed: complete response, partial response and no response. The variables included regard the categories of clinico-hematologic response (CHR, including normalization of blood counts and spleen volume, and resolution of disease-related symptoms), molecular response, and BM histology response. The rationale to maintain separate categories of response was the fact that there was no evidence that available therapies alter the natural course of disease. Therefore, while in clinical studies of new therapeutics it might be worthwhile to address the achievement of a molecular or histological response, these are not relevant in patients receiving standard treatments. In fact, the ELN criteria were developed mainly to allow for a reproducible design and interpretation of clinical trials rather than for routine practice. Most patients with PV receiving conventional treatment at best fulfill the criteria of partial response,[76] although most of those treated with interferon-α may achieve a CHR (but not necessarily resolution of splenomegaly and/or symptoms) and some also achieve a molecular response. In a retrospective study of PV patients managed with hydroxyurea and followed for four years, no association was seen between achievement of an ELN response and survival or vascular complications.[76] These findings, although biased by the retrospective characteristics and the small size of the study population, raise concerns about the real-life impact of the ELN response criteria, and identify the need for their prospective evaluation, as well as a search for more powerful surrogate markers of clinical benefit.

Interestingly, in the above cited MPN landmark study, the most important treatment goals reported by patients were slowing/delaying disease progression (25%), prevention of thrombosis (24%), normalization of normal blood counts (18%), better quality of life (12%), symptomatic improvement (9%) and maintaining a hematocrit level less than 45% (6%).[64]

Evolving concepts in the treatment of patients with PV

The basic concept: risk-adopted cytoreductive therapy

The current treatment recommendations for patients with PV rely on a few randomized studies and several consensus/clinical practice indications. The first objective of treatment is to reduce the hematocrit and associated blood viscosity to minimize the risk of thrombosis. In a seminal observational study, Pearson reported that the incidence of thrombosis directly increased with hematocrit above a level of 45%.[77] This study claimed this level of hematocrit to be the optimal target for management, but a survey of the practice patterns of American Society of Hematology members revealed that, in practice, such a hematocrit threshold was used by only a minority of physicians while 16% preferred to adopt a target of 50%.[78] It took almost 40 years to make the transition from an observation/recommendation to an evidence-based guideline. The Cyto-PV study randomly assigned 365 PV patients, irrespective of risk category (approx. one-third were low-risk) and treatment (phlebotomy, hydroxyurea, or both), to a target level of less than 45% or 45%-50%.[79] Results indicated that patients in the higher hematocrit level had a 4-times increased rate of death from cardiovascular events in comparison to those maintained at less than 45%.[79] A lower hematocrit level (ideally <42%) may be indicated (but not formally proven) in women[80] and/or in cases of SVT, where RBC volume expansion is masked by hemodilution.

How to maintain the target hematocrit level depends on the risk category. For low-risk patients, phlebotomy is still the cornerstone of treatment. For patients at high risk, cytoreduction with hydroxyurea or interferon-α is recommended. Cytoreduction is also indicated in low-risk patients to control progressive leukocytosis (no threshold formally identified) and thrombocytosis (usually above 1-1.5 million/mm^3), symptomatic splenomegaly and/or disabling symptoms. In the non-randomized PVSG-08 study[81] that included 51 treatment-naïve patients, use of hydroxyurea was associated with a significantly lower rate of thrombosis compared with the phlebotomy arm of the PVSG-01 study (6.6% *vs.* 14% at 2 years).[40]

There is growing interest in the use of interferon-α as first-line agent. The mechanisms by which interferon-α induces responses in PV have not yet been clarified. Interferon has pleiotropic activities, including effects on immune modulatory cells, inhibition of apoptosis, induced expression of pro-apoptotic genes, a direct anti-proliferative effect on hematopoietic progenitor and possibly stem cells (reviewed by Kiladjian *et al.*[82]). The efficacy of interferon-α in inducing hematologic remission in PV was first reported in 1998[83] (up-dated by Silver[84]) and confirmed in several small studies.[82] Two larger independent studies[85,86] and one sponsored[87] phase II study with different preparations of pegylated interferon-α were reported. These studies confirmed drug efficacy in inducing prompt and sustained hematologic responses, eventually associated with improvement of symptoms and splenomegaly. Furthermore, in most patients, a substantial decrease of *JAK2* mutant burden, usually after the first year of treat-

Table 6. Definition of resistance/intolerance to hydroxyurea in polycythemia vera according to the ELN consensus.[92]

1. Need of phlebotomy to keep ht <45% after 3 months of at least 2 g/day of hydroxyurea, OR
2. Uncontrolled myeloproliferation, i.e. platelet count >400x10^9/L AND WBC >10x10^9/L after 3 months of at least 2 g/day of hydroxyurea, OR
3. Failure to reduce massive* splenomegaly 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 hydroxyurea, OR
4. Absolute neutrophil <1x10^9/L OR platelet count <100x10^9/L OR hemoglobin <100 g/L at the lowest dose of hydroxyurea required to achieve a complete or partial clinico-hematologic response#, OR
5. Presence of leg ulcers or other unacceptable hydroxyurea-related non-hematologic toxicities, such as mucocutaneous manifestations, gastrointestinal symptoms, pneumonitis or fever at any dose of hydroxyurea

ELN: European LeukemiaNet; Ht: hematocrit; WBC: white blood cell count. *Organ extending by more than 10 cm from the costal margin. #Complete response was defined as: hematocrit less than 45% without phlebotomy, platelet count ≤400x10^9/L, WBC≤10x10^9/L or under, and no disease-related symptoms. Partial response was defined as: hematocrit less than 45% without phlebotomy, or response in three or more of the other criteria.

ment and including also complete responses, was documented, although this was not found in other studies.[88,89] Toxicity is lower with pegylated over conventional preparations of interferon, and a mono-pegylated interferon that requires less frequent administrations might improve tolerability.[87] However, approximately 20% of the patients stop interferon in the first year due to toxicity. Interestingly, interferon-α is not approved for the treatment of PV. Two randomized studies are ongoing, one with interferon-α 2a (sponsored by the Myeloproliferative Disorders-Research Consortium; registered at *clinicaltrials.gov identifier: 01259856*), the other with pegylated interferon-α 2b (the company sponsored PROUD-PV study; registered at *clinicaltrials.gov identifier: 1949805*), both compared with hydroxyurea. Results of these studies might eventually provide the missing evidence to support an evidence-based use of interferon as first-line agent; hopefully this will lead to its approval for clinical use.

Second-line therapy: JAK2 inhibition

Most patients do pretty well with hydroxyurea for the entire duration of their disease; however, 15%-20% develop some intolerance or become resistant to the drug over time.[90,91] A set of consensus criteria are commonly used to identify resistant or intolerant patients.[92] The development of cytopenias at the lowest dose of hydroxyurea needed to achieve a response was retrospectively associated with an increased risk of death and transformation to PPV-MF and AL.[90]

Patients who are not adequately controlled with therapy or who develop an intolerance do not have many options. In the case of intolerance, one common practice approach is to reduce the daily dose to the best tolerated one and make more generous use of phlebotomies to maintain the target hematocrit. However, too many phlebotomies are not well tolerated and may cause symptomatic iron deficiency. One may switch from hydroxyurea to interferon, although this is not supported by formal studies; *vice versa* hydroxyurea can be used if interferon is not tolerated or effective. The use of an alternative cytotoxic agent must be evaluated carefully, particularly in younger subjects, due to the increased risk of leukemia associated with alkylating agents after hydroxyurea.[42,43] Recently, the JAK1 and JAK2 inhibitor ruxolitinib has been approved for the treatment of patients with PV who are refractory to, or intolerant of, hydroxyurea based on the results of the phase III RESPONSE trial that enrolled PV patients with baseline splenomegaly and phlebotomy dependence.[73] The study demonstrated superiority of ruxolitinib *versus* best-available therapy (BAT) in controlling hematocrit without phlebotomy and reduction of enlarged spleen volume (the composite primary end point of the study, reached by 22.7% *vs*. 0.9% of the patients); hematocrit and spleen volume responses were maintained in 89% and 98% of patients, respectively, at a median of 111 weeks of exposure.[93] Less phlebotomies were required in the ruxolitinib arm to maintain hematocrit less than 45%, and the number of cardiovascular events was lower (1.8 *vs.* 8.2 in BAT per 100 patient-years); however, this end point was not statistically controlled, and interpretation of the findings remains problematic. These results were largely in line with those of the phase II study[66] and have been further confirmed in the phase III RESPONSE II study that enrolled patients with similar characteristics but without palpable splenomegaly.[94] Patients receiving ruxolitinib had significant improvement of the MPN-SAF total symptom score that concerned all individual symptoms related to splenomegaly, inflammatory cytokines and microvascular abnormalities, unlike patients receiving BAT who experienced no change or even a worsening of symptoms. Treatment was usually well tolerated with 82.7% of patients initially randomized to ruxolitinib still on therapy at the 80-week update.[93] However, patients receiving ruxolitinib experienced more frequent reactivation of Herpes Zoster infections (6.4% *vs*. 0 in BAT, mostly grade 1-2) and more non-melanoma skin cancers (4.4 *vs*. 2.0 in BAT *per* 100-patient years; however, most cases developed in patients with prior history of skin cancers), indicating that active surveillance is required in daily practice and in long-term follow-up analysis.

The unmet needs and perspectives for the future

Diagnostic criteria: too relaxed, too selective?

Early diagnosis of PV is of the utmost importance to minimize the risk of thrombosis through the prompt adoption of measures to control hematocrit, institution of antiplatelet therapy, and correction of cardiovascular risk factors. Thanks to the availability of genetic tests for *JAK2* mutation, and the revised WHO 2016 criteria, early diagnosis is possible. Although the use of the lower threshold levels of hemoglobin set by the 2016 criteria might result in some inappropriate investigations in subjects with modest, yet sustained, increased hemoglobin without obvious reason, "*Paris is worth a mass*", and potentially preventing thrombosis with prompt institution of therapy justifies the additional costs.

How to manage the risk of disease progression

Due to a greater knowledge of disease pathophysiology, earlier diagnosis and improved management, it might be assumed that the median survival of patients with PV will continue to improve mainly because of a reduction of life-threatening thrombosis.[36,56] Conversely, disappointingly, the rate of progression to PPV-MF or AML/MDS has remained unchanged over the years. Although some clinical and molecular (p53,[95,96] *IDH1* and *2* mutations[97,98]) variables have been associated with increased risk of PPV-MF and AML/MDS, none is specific enough to be clinically useful. Furthermore, it has been argued that, because of the intrinsically low pace of progression, development of PPV-MF may occur well before the worsening of fibrosis to the grade 2 or more required by IWG-MRT criteria.[99] There is, therefore, the possibility that appropriate treatment, including JAK inhibitors or stem cell transplantation, is delayed in some patients. Given this, diagnosis of PPV-MF should not be restrained by the degree of fibrosis, and novel diagnostic criteria, ideally supported by hitherto unknown biomarkers, are needed. The genetic profile of AL after PV differs from *de novo* leukemia for the notable absence of typical abnormalities, including *FLT3* and *NPM-1*. Survival from post-PV AL is dismal, with a median of 3-5 months from diagnosis,[100,101] and no medical therapy, including induction chemotherapy and ruxolitinib, provided evidence of efficacy,[102,103] although stem cell transplantation is a curative option in a few patients. Therefore, understanding the molecular basis of transformation that will help identify surrogate markers and develop effective therapeutic strategies represent urgent unmet needs. Notably, very few clinical studies have been conducted, or indeed planned, in this clinical setting.

The trouble with inheritance in a somatic disease

An extensive family history should be obtained during the diagnostic workup in any PV patient. The knowledge that PV, like other MPNs, may cluster in the family is a cause of great concern for parents of PV patients. Increased knowledge of the genetic basis of MPN and screening of family members might in the future allow early disease phases to be identified. However, at present, parents should be discouraged from performing unnecessary tests in otherwise healthy offspring, including driver mutations or germline variants such as the 46/1 allele.

Who is the "patient in need of treatment"?

The risk-adopted criteria in use for therapy do not formally account for additional variables that impact on thrombosis rate, beyond history and age, as well as for the residual approximate 2-fold risk over controls in conventionally low-risk patients. Quantitative assessment of the symptomatic burden might allow patients to be better categorized and has been widely used in clinical trials. However, how to implement these tools in practice and how to use such information for therapeutic decision making remain challenging issues. Therefore, it is urgent to develop a definition of patients with "inadequately controlled disease" who need to be shifted to second-line treatment, including ruxolitinib, a highly effective, but costly, therapy.[104] Patients who continue to have symptoms that are difficult to manage, or who manifest progressive symptomatic splenomegaly or progressive leukocytosis/thrombocytosis, or develop unacceptable toxicities with their current therapy, may obviously belong to that category of patients for whom alternative treatment is required. However, the most important indicator of an inadequately controlled PV in terms of thrombosis and survival is the hematocrit level.[79] Unfortunately, there is as yet no consensus on what is the "acceptable" rate of phlebotomies required, either alone (in low-risk patients) or combined with cytoreduction (in high-risk patients) to maintain the target level of hematocrit. Furthermore, it was shown that patients with phlebotomy requirements present a substantial symptomatic burden.[67] Just based on the ELN criteria, need of (any) phlebotomy after three months on an optimal dose of hydroxyurea would *per se* be evidence of resistance to treatment, and therefore of an inadequately controlled disease.[92] However, there is no hard evidence that this concept might be translated into the clinical practice. Timely action from the scientific community to develop consensus criteria of what constitutes an "inadequately controlled PV" is needed.

Antithrombotic prophylaxis: low-dose aspirin, anticoagulation, or both?

Evidence from the ECLAP trial[55] led to the recommendation of low-dose aspirin in all PV patients (unless contraindicated) and this has certainly contributed to the improvement in outcome that has been observed since. However, the rate of recurrent thrombosis, and the residual risk in low-risk patients, remain unsatisfactorily high, and should prompt studies of novel approaches for both primary and secondary prophylaxis. From studies in non-PV patients, it might be assumed that twice daily aspirin is more efficacious against arterial, and possibly venous, thrombosis, than once daily, but this still has to be proved, as does the added value of combination therapy with oral anticoagulants in patients with a history of venous events. The duration of anticoagulant prophylaxis in certain conditions, such as SVT or pulmonary embolism, the added value of statins, and the role of new direct oral anticoagulant are all questions that need to be addressed in prospective studies.[105]

Do we want or need molecular remission?

The BCR-ABL negativization produced by imatinib and other TKIs in chronic myelogenous leukemia represents the holy grail for PV, too. However, it must be emphasized that reduction/elimination of the *JAK2*V617F allele might not necessarily be indicative of cure, since other mutated clones preceding *JAK2*V617F acquisition, and also hematologic abnormalities, may persist even in patients with complete molecular remissions induced by interferon[106] and ruxolitinib.[107] Therefore, while elimination of *JAK2*V617F mutated cells certainly constitutes a goal for novel therapies, the impact of molecular responses on the natural history of disease remains uncertain and further studies are required.

What's available?

Ruxolitinib is the first and only JAK2 inhibitor approved for second-line treatment in PV; a phase II study with another JAK2 inhibitor, momelotinib, is ongoing (*clinicaltrials.gov identifier: 019898828*). An alternative class of potentially active drugs is made up by the histone deacethylase inhibitors. Vorinostat was poorly tolerated, with 44% of patients discontinuing treatment early.[108] Givinostat proved promising in a phase II study for control of hematocrit and symptoms,[109] and was also tested in combination with hydroxyurea in patients who were refractory to this drug, producing responses in approximately half.[110] A phase Ib/II study to assess the safety and tolerability, and preliminary efficacy, in PV patients (*clinicaltrials.gov identifier: 01901432*) is ongoing. Overall, not much is available and the shelf is quite empty. Novel molecular insights are urgently needed to boost pharmacological research.

Personalized medicine for PV: "what's in a word?"

After the discovery of aberrantly activated JAK/STAT signaling as the basic pathogenetic defect, PV has potentially entered the arena of personalized medicine. However, it remains uncertain how to transfer this new information into the daily management of the individual patient. Current therapeutic decisions are dictated by the needs of the individual patient and/or are based on predictive clinical variables, such as age and history of thrombosis that are not 'druggable', rather than by the disease itself. There is some evidence of a 'cure', but we still do not know how big an impact it might have on long term-outcome, as compared to optimized disease management, or how expensive it would be (in terms of side effects and money) to get it. Since new drugs may be more effective than conventional ones, but are not without toxicity and are costly, predictive biomarkers need to be identified. In the meanwhile, fostering basic research, producing evidence-based data and developing evidence-based recommendations seem to be the most productive approach to move towards personalized management of patients with PV.

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