**Myeloproliferative disorders**

**Diagnosis and treatment of polycythemia vera and essential thrombocythemia**

A.M. Vannucchi

Section of Hematology, Department of Critical Care, University of Florence and Istituto Toscano Tumori, Florence, Italy

Hematology Education: the education programme for the annual congress of the European Hematology Association

2011;5:255-263

**Introduction**

Landmark discoveries occurred in the last few years have greatly advanced our knowledge of pathophysiology and contributed to a reassessment of the diagnostic criteria of polycythemia vera and essential thrombocythemia. On the other hand, the prognostic relevance of mutation abnormalities is still largely debated and very likely it is overall modest. Thus, the criteria employed for patients risk stratification are still exclusively based on clinical considerations. On the other hand, we are witnessing a remarkable number of ongoing clinical trials with drugs belonging to different classes that include interferon, inhibitors of histone deacetylases and JAK2 inhibitors, which hold the promise to improve the therapeutic approach to at least selected categories of patients.

Polycythemia vera (PV) and essential thrombocythemia (ET) are stem-cell derived clonal myeloproliferative neoplasms (MPN) that have only recently witnessed significant advances in the understanding of their molecular basis. A landmark discovery occurred in 2005, with the identification of a recurrent point mutation in exon 14 of JAK2, the gene encoding the type I receptor-associated JAK2 tyrosine kinase. JAK2 is implicated in the intracellular transduction of signals originating from numerous cytokine receptors predominantly through the STAT pathway. However, the molecular complexity of MPN was surprisingly greater than anticipated, and the number of mutations being discovered has increased steadily in the last couple of years, on the other hand, the hierarchy of molecular abnormalities and their original cell target remain largely to be defined. Prompted by these seminal discoveries, experts of the 2008 WHO classification subcommittee revised the terminology underlying the “neoplastic” nature of the “myeloproliferative disorders”, as they had been named after William Dameshek, and at the same time, refined and improved the diagnostic criteria for the three “classic” MPN that include PV, ET, and primary myelofibrosis (PMF). Finally, in an exceptionally short lapse of time thereafter, results of the first clinical trials employing small-molecule inhibitors that target JAK2 (and JAK1) became available, providing proof-of-concept of the effectiveness of a targeted-therapy. In this review, I will briefly present the current approach to the diagnosis and treatment of PV and ET aiming at illustrating, whenever possible, how novel molecular information resulted in modification of our clinical approach.

**The diagnosis of PV and ET**

The detection of a JAK2 V617F mutation in at least 95% of PV and 60% of ET patients has made diagnosis of these disorders easier and more accurate than in the “pre-JAK2” era, and is at the basis of the WHO 2008 revision of the diagnostic criteria (Table 1). Indeed, when the criteria for defining a raised hemoglobin level are satisfied, the presence of JAK2 V617F mutation and subnormal serum erythropoietin levels support the diagnosis of PV with virtually absolute specificity, and distinguish it from conditions associated with reactive increase of hemoglobin. When erythropoietin levels are subnormal but the V617F mutation is absent, then searching for mutations in JAK2 exon 12 is recommended; the latter allow us to characterize molecularly a further approximate 2% of patients with V617F-negative PV. In a recent European collaborative study that recruited 106 JAK2 exon 12-mutated PV patients, a total of 17 different mutations were identified. JAK2 exon 12 mutated patients had significantly higher hemoglobin level and lower platelet and leukocyte count at diagnosis compared with JAK2V617F-mutated PV subjects; two-thirds of the patients manifested isolated erythrocytosis only. The incidence of thrombosis, myelofibrosis, or leukemia, and the overall survival, were similar to JAK2V617F mutated subjects. Mutations in LNK, a plasma membrane-bound adaptor protein that inhibits phosphorylation of wild-type and mutant JAK2, were originally described in one ET and PMF patient by Oh and colleagues; however, functional defects of Lnk have been found in a large proportion of MPN patients also in the absence of mutations. Finally, LNK mutations have been recently described also in two of eight JAK2-wild-type PV patients who manifested isolated erythrocytosis without other clinical, laboratory, or bone conditions.
marrow morphologic features of classic PV.

The diagnostic approach to patients presenting with thrombocytosis as the main hematologic abnormality is indeed more cumbersome; as a matter of fact, the reliability of some histologic criteria included in the WHO classification employed for differential diagnosis has been questioned. Disorders that mimic ET include reactive thrombocytosis, PMF, EEC, and refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T), as well as other unusual chronic myeloid neoplasms. The JAK2 V617F mutation is harbored by approximately 60% of the patients who are finally diagnosed as ET, while an additional 5–8% present mutations in MPL exon 10 at codon 515. When these clonal markers are present, they reliably exclude the possibility of reactive thrombocytosis, while negativity for BCR-ABL rules out CML; in RARS-T, JAK2 V617F mutation is present in more than 50–60% of cases and is invariably associated with signs of dyserythropoiesis. Thus, at the present time, at least 30–40% of ET patients remain molecularly not characterized, and according to the WHO criteria, the diagnosis most relies on bone marrow histology; the degree of trilineage proliferation, megakaryocyte morphology, and topography, and the extent of fibrosis are employed as key variables in distinguishing ET from prodromal stages of PV and PMF.

A particularly debated issue concerns the distinction between so-called “true ET” and “prefibrotic myelofibrosis”. Overt fibrosis is absent in bone marrow biopsy of prefibrotic myelofibrosis, possibly leading to a spurious diagnosis of ET. In order to contribute to solving these issues, in a recent international study, 1,104 bone marrow biopsies from patients diagnosed as ET in seven experienced centers were collected and centrally re-reviewed by one author of the WHO classification, with the aim to ensure strict adherence to the WHO histologic criteria for diagnosis. It was found that the overall survival and the rate of transformation to leukemia and to overt myelofibrosis were significantly worse in early/prefibrotic myelofibrosis compared with ET patients, while thrombotic complication rates were similar. However, whether “early/prefibrotic myelofibrosis” and “true ET” are two different entities or rather they reflect distinct evolution stages of a single disease remain to be clarified. The negative impact of reticulin accumulation at diagnosis has been demonstrated in a large series of ET patients from the PT-1 trial. Elevated reticulin levels predicted higher rates of arterial thrombosis, major hemorrhage, and myelofibrotic transformation independently of known risk factors. Elevated reticulin levels at presentation predicted higher rates of arterial thrombosis (hazard ratio [HR], 1.8; 95% CI, 1.1 to 2.9; P = .01), major hemorrhage (HR, 2.0; 95% CI, 1.0 to 3.9; P = .05), and myelofibrotic transformation (HR, 5.5; 95% CI, 1.7 to 18.4; P = .0007) independently of known risk factors.

The International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) has developed criteria for diagnosing myelofibrotic evolution of PV and ET.

### Therapeutic approach to ET and PV

#### Clinical needs of patients with PV and ET

PV and ET are relatively indolent disorders which, according to most studies, result in a modest reduction of survival, usually after the first decade from diagnosis. However, in a recent retrospective study from the Swedish Cancer Registry that included 4,389 and

<table>
<thead>
<tr>
<th>Table 1. The WHO criteria for diagnosis of PV and ET.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polythemia vera</strong></td>
</tr>
<tr>
<td><strong>Major criteria</strong></td>
</tr>
<tr>
<td>1. Hgb &gt; 18.5 g/dL (men) or &gt; 16.5 g/dL (women)</td>
</tr>
<tr>
<td>Hgb or Hct &gt; 99th percentile of reference range for age</td>
</tr>
<tr>
<td>or</td>
</tr>
<tr>
<td>or</td>
</tr>
<tr>
<td>Hgb &gt; 17 g/dL (men) or &gt; 15 g/dL (women) if associated</td>
</tr>
<tr>
<td>with a sustained increase of ≥ 2 g/dL from baseline that cannot</td>
</tr>
<tr>
<td>be attributed to correction of iron deficiency</td>
</tr>
<tr>
<td>or</td>
</tr>
<tr>
<td>Elevated red cell mass &gt; 25% above mean normal predicted value</td>
</tr>
</tbody>
</table>

Minor criteria
- 2. Presence of JAK2V617F or similar mutation
- 3. EEC growth

Diagnostic combinations
- Both major criteria + 1 minor criterion
- First major criterion + 2 minor criteria

Epo, erythropoietin; EEC, endogenous erythroid colonies; LDH, lactate dehydrogenase.
2,559 patients with a diagnosis of PV and ET, respectively, a significant overall excess mortality compared with reference population was found.18 The relative survival ratio (RSR) at 10 years was 0.64 (95% CI, 0.62–0.67) for PV and 0.68 (0.64–0.71) for ET. There was a trend towards improved survival in the last decade, mostly ascribable to a reduction in the number of deaths due to cardiac disorders and thromboembolic events; thus, the commonest causes of death were represented by hematological malignancies and solid tumors. Evolution to post-polycythemic or post-thrombocytopenic myelofibrosis (PPV-/PET-MF) and transformation to acute myeloid leukemia (AML) contributed heavily to reduced survival.

Much attention has been paid in last years to the patients’ quality of life (QoL) that is burdened by a spectrum of complications, including thrombosis, hemorrhages, constitutional symptoms, fatigue, pruritus, microvascular manifestations, and increased risk of miscarriage. Also the side effects of treatment, such as phlebotomy-induced iron deficiency or the mucous and skin toxicities due to hydroxyurea, can contribute to an overall reduced QoL. In an Internet-based symptom survey of 1,179 patients with MPN, of whom 405 were PV and 304 were ET, more than 70% of the patients reported symptoms ascribable to the underlying disease: fatigue in 72–85%, night sweats in 40–49%, and bone pain in 40–45%. Pruritus was prevalent in PV (65% vs. 39% in ET).39 To improve and standardize the measurement of QoL-related issues, Mesa et al. recently developed an internationally validated Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) that complements and extends the SAF previously developed specifically for Myelofibrosis (MSAF).40–41 The MPN-SAF contains 27 items that address symptoms related to spleen enlargement, myeloproliferation, vascular events, manifestations due to abnormally increased pro-inflammatory cytokine levels, fatigue, and psychiatric aspects. It is expected that this instrument will be an essential part of any novel clinical trial and will permit us to assess reproducibly the response of disease-associated symptoms to novel (and conventional) therapies.

These patients’ “clinical needs” represent the goals of therapy in PV and ET that, according to the management recommendations recently developed by the European Leukemia Net (ELN), are: to avoid first occurrence and/or recurrence of thrombotic and/or hemorrhagic complications; to minimize the risk of evolution to PPV-/PET-MF or transformation to AML; to control systemic symptoms; and to optimally manage potentially risky situations, such as surgery or pregnancy.42

**Indications for treatment based on risk stratification**

Established risk factors for reduced survival in PV and ET include advanced age and history of cardiovascular events. Leukocytosis and anemia have also been reported to impact on survival negatively, but they have not been validated prospectively yet.43,44 Considering that therapy is not curative, the rationale behind current risk stratification in PV and ET is based on an estimate of the risk of thrombotic complications. Age older than 60 years and a history of thrombosis are the criteria used to classify patients into a “high-risk” (when either of these is present) or “low-risk” (when both are absent) category. In a cohort of 1,638 PV patients who were screened as part of the European Collaboration on Low-Dose Aspirin (ECLAP) trial, the rate of events was 2.5 per 100-patients/year among low-risk subjects compared with 10.9 per 100-patients/year among those who were older than 65 and had a prior thrombosis.45 The role of additional, generic, risk factors for thrombosis (diabetes, obesity, hypertension) is not clearly defined, and whether subjects presenting such abnormalities configure an “intermediate-risk” category is not currently supported by evidence. Smoking is associated with an increased risk of arterial thrombosis in PV,46,47 and should be strongly discouraged in PV and ET patients; avoidance of hormonal therapy in women is recommended. On the other hand, “extreme” thrombocytoysis (where “extreme” means >1,000x10^9/L platelets for some and >1,500x10^9/L for others) is considered a risk factor for hemorrhages, possibly due to an acquired von-Willebrand like disorder,48,49 but it is not associated with an increased rate of thrombosis. Paradoxically, in a retrospective study in ET, a platelet count greater than 1,000x10^9/L was found to exert a protective effect on thrombosis,48 thus supporting a number of previous evidences that thrombosis is not directly associated with high platelet count (reviewed in 18). A correlation between thrombosis and leukocytosis,47,52–57 JAK2 mutated genotype, or the JAK2V617F allelic burden58–60 is suggested in some studies but denied in others; therefore, leukocytosis and JAK2 mutational status are not included in current risk stratification and should not guide therapy. However, due to their possible relevance for the pathogenesis of thrombosis and as “surrogate” end-points for therapy, it would be very important that their significance is addressed in prospective controlled studies.

**Conventional treatment of ET and PV**

The cornerstones of the management of patients with ET and PV are the control of (i) erythrocytosis, through phlebotomies, alone or in association with cytoreductive drugs; (ii) thrombocytosis, through the use of platelet-lowering agents; and (iii) abnormal platelet function by antiplatelet therapy with aspirin. Indications vary according to the patient’s risk category (Table 2). It should be pointed out that these indications represent shared opinions among experts but evidence based on clinical trials has been produced only for the use of low-dose aspirin in patients with PV61 and for hydroxyurea in high-risk patients with ET.62,63

Low-risk patients with PV are managed only with therapeutic phlebotomies while in high-risk patients with PV and ET, cytoreduction is recommended. In PV and ET patients, low-dose aspirin (81 to 100 mg daily) should be prescribed independent of the risk category; however, in the youngest, asymptomatic patients with ET who do not have additional generic cardiovascular risk factors, a watch-and-wait attitude is justified as well. Conversely, due to the risk of bleeding, aspirin must be used with caution in the presence of extreme thrombocytoysis or other relevant contraindications (allergy, asthma, previous severe gastric bleeding, laboratory evidence of acquired von Willebrand disease in subjects with a history of hemorrhagic manifestations). Whether
anti-aggregants other than aspirin offer the same protection against thrombosis remains to be evaluated in clinical trials. Cytoreduction is sometimes employed in low-risk patients for a number of reasons that include poor tolerance to, or too high frequency of, phlebotomies (in case of PV); symptomatic splenomegaly; evidence of progressive myeloproliferation manifested by leukocytosis or extreme thrombocytosis; presence of severe constitutional symptoms; and/or pruritus. Generic cardiovascular risk factors are usually not a reason for treating otherwise low-risk patients, and decision in this regard should be individualized; however, those risk factors should be corrected with diet, physical exercise, anti-diabetics, anti-hypertensive drugs, lipid lowering agents, as more appropriate.

It is usually recommended that the hematocrit target is set at less than or equal to 45% and less than or equal to 42% in men and women with PV, respectively. However, these targets derived mainly from a small trial and have not been substantiated rigorously. As a matter of fact, a large retrospective study in Europe found no difference in the rate of thrombosis or death in patients who had their hematocrit maintained under 45% or above 45% (up to 55%) and received concomitantly low-dose aspirin. A large prospective trial (CYTOPV) that compares two different hematocrit targets (level of 45–50%) is under way in Italy. Similarly, there is no based evidence to support the commonly chosen target level of 400 x 10^9/L platelets in high-risk patients with ET.

Drugs currently employed as first-line therapy are represented by hydroxyurea and interferon-α in countries where it is available to such a purpose, although it is not approved by FDA or EMA. Hydroxyurea is usually started at 500–1000 mg/day, and the dose is titrated based on the target level of hematocrit and/or platelet count. The drug is usually well-tolerated, but gastrointestinal intolerance, mucous, or cutaneous ulcers, skin toxicity, fever, or in rare instances, pulmonary can limit its use in individual patients. Conventional formulations of INF-α have been successfully employed to control hematocrit in 50–94% of PV patients or platelet count in greater than 70–80% of ET patients (reviewed in). However, INF-α has severe side effects leading to discontinuation in more than 30–40% of the patients, including fatigue, flu-like syndrome, worsening or developing of autoimmune diseases, psychiatric manifestations, and myelosuppression. More recently, pegylated forms of INF-α have been developed to increase drug half-life and to reduce the frequency of administrations. In two recent studies, pegylated INF-α2a, usually at 90 μg/week, was employed in PV (40 patients) or in PV and ET (40 and 39 subjects, respectively). Greater than 80% of the patients obtained hematologic remission accompanied by a continuous decrease of JAK2 V617F allele burden; up to 10% of the subjects achieved the complete disappearance of measurable JAK2V617F allele. On the other hand, about 20% of the patients had to discontinue the treatment due to side effects. As a whole, results of these two studies confirm the hematologic efficacy of INF-α in PV and ET and the potential for achieving the eradication of JAK2V617F mutated cells (which could not correspond to eradication of the disease, according to a recent report that demonstrated persistence of TET2 mutated cells in some patients who became JAK2V617F-negative after interferon). A controlled randomized study of pegylated INF-α2a versus hydroxyurea in high-risk patients with PV and ET has been launched recently by the MPD-RC group, and will hopefully provide definite conclusions about the superiority of interferon to hydroxyurea as first-line therapy for PV or ET. A second study of the MPD-RC is a single arm salvage therapy with pegylated INF-α2a in high-risk PV or ET patients who are resistant or intolerant to hydroxyurea or have had splanchic thrombosis.

If hydroxyurea is ineffective, poorly tolerated, or causes significant toxicity, the drugs commonly employed as second-line therapy are represented by INF-α, particularly in young patients, busulfan, pipobroman, or anagrelide in case of ET. Radiophosphorus is rarely employed in older PV patients, and in studies of the Polycythemia Vera Study Group (PVSG) in the 1980s, this treatment was associated with an increased rate of leukemiation transformation. Busulphan is preferred in older patients; the drug needs careful titration because of its potent myelosuppressive effects. Anagrelide is approved in Europe as second line for hydroxyurea resistant or intolerant patients with ET. A large randomized trial, the FT-1, that compared hydroxyurea with anagrelide on the top of low-dose aspirin, in high-risk ET patients concluded for an overall superiority of hydroxyurea due to a significant less risk of arterial thrombosis, major hemorrhages, and fibrotic transformation, although anagrelide proved superior against venous thrombosis. In a recent randomized study, anagrelide was not inferior to hydroxyurea as a single drug in the treatment of newly-diagnosed high-risk ET patients; however, results of this study should be interpreted with caution because of the low statistical power due to a “non-inferiority” trial design. Side effects of anagrelide include headache, flushing, cardiopalm, and arrhythmias, and led to discontinuation in a greater proportion of the patients than hydroxyurea in the above mentioned trials. Due to the anti-platelet activity of anagrelide, the concominient profit of other...
tant use of aspirin should be carefully evaluated.

One still largely debated issue about the use of hydroxyurea concerns its leukemic potential; however, this is not substantiated by any of the clinical trials available to date, although it should be acknowledged that none of these was specifically designed to this endpoint. Conversely, the combined use of multiple chemotherapeutic agents has been associated with a higher rate of leukemic transformation than expected. Leukemia is part of the natural history of these disorders, as supported by the observation that the rate of evolution to leukemia among PV or ET patients who were un-treated or had received hydroxyurea only was 7.4% and 3.3%, respectively. In another study that included three French prospective trials with hydroxyurea and pipobroman, the rate of leukemia transformation was 12 to 15%, with an excess for pipobroman. Most cases of transformation occurred after 15 years, and there was no evidence of a plateau. A standardized definition for clinical resistance and intolerance to hydroxyurea in ET and PV was developed by a group of experts of the ELN using consensus methodologies (Table 3). These criteria can be conveniently used for decision-making when assessing the opportunity to move patients to second-line therapies and/or for identifying those suitable for enrollment in clinical trials with novel drugs. Furthermore, criteria for monitoring the response to treatment in PV and ET have been developed by the ELN group (Table 4). These criteria involve the measurement of levels of response and their ranking according to three sets of categories: clinical-hematological, molecular, and histological response. Presently, only clinical-hematological criteria should be employed for monitoring the response to conventional cytoreductive therapy, since no drug, with the exception of interferon, has produced yet relevant effects on mutated allele burden. In fact, JAK2 V617F allele burden is not influenced even in the absence of any treatment, and claims of significant decline of JAK2V617F allele burden in patients treated with hydroxyurea have not been universally reproduced. Thus, sequential monitoring of molecular response can be recommended only in the settings of clinical trials and not in routine management; conversely, there is no indication to perform serial bone marrow evaluations if not clinically indicated.

### Novel drugs

A part for interferon, two novel categories of drugs, the "JAK2" inhibitors and the histone deacetylase inhibitors, have recently been evaluated in PV and ET and preliminary reports have been presented. INCB018424 is a JAK1 and JAK2 inhibitor that has been first used in a clinical trial including 153 patients with primary or PPV/PET-MF. The drug was overall well tolerated, with few and low-grade toxicities; most common side effects were due to on-target activity and included reversible thrombocytopenia (that represents the dose limiting toxicity) and anemia, that could be managed with dose titration. As many as 44% of the evaluable patients presented a greater than or equal to 50% reduction of spleen enlargement, and more than 80% experienced significant improvement of constitutional symptoms, including pruritus, night sweats, early satiety, abdominal discomfort, and fatigue. These effects are probably due to a normalization of increased proinflammatory cytokines mediated by the anti-JAK1 activity. The drug has also been employed in a phase 2 trial in patients with PV and ET. This study included 39 subjects with ET and 34 with PV who were intolerant/refractory to hydroxyurea (ELN criteria). The overall response rate was 97% (50% complete and 47% partial) in PV and 90% (26% complete and 74% partial) in ET. In PV, 97% of the patients achieved control of hematocrit to less than 45% in the absence of phlebotomies, and 68% experienced a complete resolution of enlarged spleen; more than 70% of patients with leukocytosis or thrombocytosis at baseline normalized their blood count. Among ET patients, 49% achieved a normal platelet count while 79% reached a platelet count less than 600 x 10^9/L or a decrease greater than 50% at last follow-up visit. In 13 of 14 patients

---

**Table 3. European LeukemiaNet criteria of resistance or intolerance to hydroxyurea in patients with PV and ET.**

**PV:**

1. Need for phlebotomy to keep hematocrit <45% after 3 months of at least 2 g/day of HU, OR
2. Uncontrolled myeloproliferation, i.e., platelet count >400 x 10^9/L AND white blood cell count >10 x 10^9/L after 3 months of at least 2 g/day of HU, OR
3. Failure to reduce massive splenomegaly by more than 50% as measured by palpation, OR failure completely to relieve symptoms related to splenomegaly, after 3 months of at least 2 g/day of HU, OR
4. Absolute neutrophil count <1.0 x 10^9/L OR platelet count <100 x 10^9/L or hemoglobin <100 g/L at the lowest dose of HU required to achieve a complete or partial clinical-hematological response, OR
5. 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 HU

**ET:**

1. Platelets >600,000/µL after 3 months of at least 2 g/day of hydroxyurea (2.5 g/day in patients with a body weight >80 kg)
2. Platelets >400,000/µL and WBC less than 2,500/µL at any dose of hydroxyurea
3. Platelets >400,000/µL and Hb less than 10 g/dL at any dose of hydroxyurea
4. Presence of leg ulcers or other unacceptable mucocutaneous manifestations at any dose of hydroxyurea
5. Hydroxyurea-related fever
with platelet count greater than 1,000x10^9/L, a greater than 50% reduction was observed. Similar to the myelofibrosis trial, INCB018424 was well tolerated after a medium follow-up of 21 months. A phase 3 trial in PV, the RESPONSE trial, started to recruit patients in the first quarted of 2011. This is a randomized trial where 300 patients with PV who are refractory or intolerant to hydroxyurea will be randomized to receive INCB018424 or best available therapy for 32 weeks, with the possibility of cross-over in case of failure to reach the endpoints at that time.

Another inhibitor of JAK2 kinase activity, lestaurtinib (CEP-701), was found to inhibit in vitro the expansion of CD34+ cell-derived erythroid cells from MPN patients preferentially when compared with controls.89 This agent was tested in a cohort of 39 JAK2 V617F-positive subjects, 27 and 12 of whom had a diagnosis of “high-risk” PV and ET, respectively.90 The primary endpoint of the trial was a reduction in JAK2 V617F neutrophil allele burden; secondary endpoints included reduction in hydroxyurea dose, and improvement of spleen size. At last update, it was found that among the patients who concluded the scheduled 18 weeks of treatment more than 80% had a reduction of spleen size and amelioration of pruritus; reduction of phlebotomy rate was seen in some patients, but occurred after 6 months of therapy and was not associated with concomitant improve-

ment of white cell or platelet count. Conversely, platelet and white cell counts increased in many patients while on the drug. It was unexpected that amongst the serious adverse events that occurred there were six arterial and venous events in five patients,90 a complication that has not been reported yet in other trials with JAK1/JAK2 inhibitors in myelofibrosis or in “high-risk” PV and ET. It remains to ascertain whether these events are related specifically to the drug or simply reflect its ineffectiveness in preventing trombosis.

The orally available HDAC inhibitor ITF2357 (Givinostat) has been administered to 12 PV and 1 ET patients in a phase 2 study; also included were 16 subjects with myelofibrosis.91 The rationale underlying this study was that Givinostat was shown able to induce a specific down modulation of the phosphorylated JAK2 V617F protein and inhibition of its downstream signaling while it minimally affected the wild type JAK2 in cells lacking the JAK2 V617F mutation.92 The drug was usually well tolerated, although most patients experienced grade 2 gastrointestinal toxicity. Among the 13 PV/ET patients, 1 complete, 6 partial, and 4 no responses were documented, while 2 patients went off-study prematurely. Spleen enlargement improved in 75% of PV patients, and the majority experienced improvement of constitutional symptoms and pruritus. There was evidence of a trend towards reduction of the V617F allele burden, although the short treatment period (median of 20 weeks) precluded any firm conclu-

### Table 4. Criteria for definition of clinico-hematologic, molecular and histologic response in patients with PV and ET according to the European LeukemiaNet.

<table>
<thead>
<tr>
<th>Polycythemia Vera</th>
<th>Essential Thrombocythemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinico-hematologic response</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Complete Response</strong></td>
<td></td>
</tr>
<tr>
<td>Ht lower than 45% without phlebotomy, AND Platelet count ≤ 400 x 10^9/L, AND WBC count ≤ 10 x 10^9/L, AND Normal spleen size on imaging, AND No disease related symptoms</td>
<td></td>
</tr>
<tr>
<td>Platelet count ≤ 400 x 10^9/L, AND No disease-related symptoms, AND Normal spleen size on imaging, AND White blood cell count ≤ 10 x 10^9/L</td>
<td></td>
</tr>
<tr>
<td><strong>Partial Response</strong></td>
<td></td>
</tr>
<tr>
<td>In patients who do not fulfil the criteria for complete response: Ht lower than 45% without phlebotomy, OR Response in 3 or more of the other criteria</td>
<td></td>
</tr>
<tr>
<td>In patients who do not fulfil the criteria for complete response: Platelet count ≤ 600 x 10^9/L OR decrease &gt;50% from baseline</td>
<td></td>
</tr>
<tr>
<td><strong>No Response</strong></td>
<td></td>
</tr>
<tr>
<td>Any response that does not satisfy partial response</td>
<td></td>
</tr>
<tr>
<td>Any response that does not satisfy partial response</td>
<td></td>
</tr>
</tbody>
</table>

| **Molecular response** |
| **Complete response** |
| Reduction of any specific molecular abnormality to undetectable levels |
| **Partial Response** (Applies only to patients with a baseline value of mutant allele burden greater than 10%) |
| A reduction equal to or greater than 50% from baseline value in patients with less than 50% mutant allele burden at baseline, OR A reduction equal to or greater than 25% from baseline value in patients with more than 50% mutant allele burden at baseline |

| **No Response** |
| Any response that does not satisfy partial response |

| **Histologic response** |
| **Bone marrow histological remission** |
| Presence of age adjusted normocellularity and no reticulin fibrosis |
sions at this regard. Based on these encouraging results, a clinical study envisioning the concomitant administration of low dose Givinostat and hydroxyurea has completed enrollment as of January 2011. It is of note that both INCB18424 and Givinostat had remarkable effects on the symptomatic control of pruritus; indeed, intractable pruritus, typically aquagenic, is complained by most PV patients, and sometimes represents a disabling condition. It is very poorly responsive to conventional treatments, including antihistamines, the serotonin uptake inhibitor paroxetine, phototherapy with UVA light, and psoralen. Remissions after interferon therapy are more common.

The pathogenetic mechanisms are still largely unknown, but abnormal activation of JAK2 V617F mutated basophils and mast cells has been recently considered causative.

Conclusions

The improved understanding of the molecular pathogenesis of ET and PV that followed the seminal discovery of JAK2 V617F mutation in 2005 has rapidly translated in an improved diagnostic approach, as it is outlined in the WHO 2008 revised classification of MPN. While this is certainly true for PV, owing to the almost universal presence of a mutation in JAK2, diagnostic uncertainties still remain in approximately 40% of cases of ET negative for JAK2 V617F or MPL mutations. Also, are ET and PV two unique diseases or just one, “continuum” disease? Furthermore, the relationships between so called “true” ET and “pre-fibrotic” myelofibrosis are still largely a matter of debate that goes beyond simple classification, if the latter condition holds a worse prognosis as recent data suggest. Therefore, notwithstanding the simplification and increased robustness of current diagnostic criteria, we are not yet in the position to enlist MPN using only molecular criteria nor to use molecular signature as a valid criterion for disease entity sub-classification, patient risk stratification, therapeutic decision, or monitoring the response to therapy. Furthermore, we can reasonably expect that in the next years, a number of prospective controlled clinical trials will provide information as to whether any of the novel agents, alone or in combination, is able to improve the achievement of major therapeutic goals, that is, prevention of thrombosis, amelioration of the quality of life, and better leukemia-free and overall survival. Because of these reasons, the field of MPN will certainly continue to receive much attention and interest as it never had in the past.

References


