



Chronic Myeloproliferative Disorders

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The Philadelphia chromosome-negative chronic myeloproliferative disorders (CMPD), polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (IMF), have overlapping clinical features but exhibit different natural histories and different therapeutic requirements. Phenotypic mimicry amongst these disorders and between them and nonclonal hematopoietic disorders, lack of clonal diagnostic markers, lack of understanding of their molecular basis and paucity of controlled, prospective therapeutic trials have made the diagnosis and management of PV, ET and IMF difficult.

In Section I, Dr. Jerry Spivak introduces current clinical controversies involving the CMPD, in particular the diagnostic challenges. Two new molecular assays may prove useful in the diagnosis and classification of CMPD. In 2000, the overexpression in PV granulocytes of the mRNA for the neutrophil antigen NBI/CD177, a member of the uPAR/Ly6/CD59 family of plasma membrane proteins, was documented. Overexpression of PRV-1 mRNA appeared to be specific for PV since it was not observed in secondary erythrocytosis. At this time, it appears that overexpression of granulocyte PRV-1 in the presence of an elevated red cell mass supports a diagnosis of PV; absence of PRV-1 expression, however, should not be grounds for excluding PV as a diagnostic possibility. Impaired expression of Mpl, the receptor for thrombopoietin, in platelets and megakaryocytes has been first described in PV, but it has also been observed in some patients with ET and IMF. The biologic basis appears to be either alternative splicing of Mpl mRNA or a single nucleotide polymorphism, both of which involve Mpl exon 2 and both of which lead to impaired posttranslational glycosylation and a dominant negative effect on normal Mpl expression. To date, no Mpl DNA structural abnormality or mutation has been identified in PV, ET or IMF.

In Section II, Dr. Tiziano Barbui reviews the best clinical evidence for treatment strategy design in PV and ET. Current recommendations for cytoreductive therapy in PV are still largely similar to those at the end of the PVSG era.

Phlebotomy to reduce the red cell mass and keep it at a safe level (hematocrit < 45%) remains the cornerstone of treatment. Venesection is an effective and safe therapy and previous concerns about potential side effects, including severe iron deficiency and an increased tendency to thrombosis or myelofibrosis, were erroneous. Many patients require no other therapy for many years. For others, however, poor compliance to phlebotomy or progressive myeloproliferation, as indicated by increasing splenomegaly or very high leukocyte or platelet counts, may call for the introduction of cytoreductive drugs. In ET, the therapeutic trade-off between reducing thrombotic events and increasing the risk of leukemia with the use of cytoreductive drugs should be approached by patient risk stratification. Thrombotic deaths seem very rare in low-risk ET subjects and there are no data indicating that fatalities can be prevented by starting cytoreductive drugs early. Therefore, withholding chemotherapy might be justifiable in young, asymptomatic ET patients with a platelet count below 1,500,000/mm³ and with no additional risk factors for thrombosis. If cardiovascular risk factors together with ET are identified (smoking, obesity, hypertension, hyperlipidemia) it is wise to consider platelet-lowering agents on an individual basis.

In Section III, Dr. Gianni Tognoni discusses the role of aspirin therapy in PV based on the recently completed European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) Study, a multi-country, multicenter project aimed at describing the natural history of PV as well as the efficacy of low-dose aspirin. Aspirin treatment lowered the risk of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke (relative risk 0.41 [95% CI 0.15–1.15], $P = .0912$). Total and cardiovascular mortality were also reduced by 46% and 59%, respectively. Major bleedings were slightly increased nonsignificantly by aspirin (relative risk 1.62, 95% CI 0.27–9.71).

In Section IV, Dr. Giovanni Barosi reviews our current understanding of the pathophysiology of IMF and, in particular, the contributions of anomalous megakaryocyte proliferation, neoangio-

genesis and abnormal CD34⁺ stem cell trafficking to disease pathogenesis. The role of newer therapies, such as low-conditioning stem cell transplantation and thalidomide, is discussed in the context of a general treatment strategy for IMF. The results of a Phase II trial of low-dose thalidomide as a single agent in 63 patients with myelofibrosis with meloid metaplasia (MMM) using a dose-escalation design and an overall low dose of the drug (The European Collaboration on MMM) will be pre-

sented. Considering only patients who completed 4 weeks of treatment, 31% had a response: this was mostly due to a beneficial effect of thalidomide on patients with transfusion dependent anemia, 39% of whom abolished transfusions, patients with moderate to severe thrombocytopenia, 28% of whom increased their platelet count by more than $50 \times 10^9/L$, and patients with the largest splenomegalies, 42% of whom reduced spleen size of more than 2 cm.

I. CLINICAL CONTROVERSIES INVOLVING THE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Polycythemia vera (PV), idiopathic myelofibrosis (IMF), and essential thrombocytosis (ET) have been traditionally classified under the rubric “the chronic myeloproliferative disorders”¹ because they share the following features: involvement of a multipotent hematopoietic progenitor cell²; dominance of the transformed clone over nontransformed hematopoietic progenitor cells³; overproduction of one or more of the formed elements of the blood in the absence of a definable stimulus; growth factor-independent colony formation in vitro⁴; marrow hypercellularity; megakaryocyte hyperplasia and dysplasia; abnormalities predominantly involving chromosomes 1, 8, 9, 13, and 20^{5,6}; thrombotic and hemorrhagic diatheses; exuberant extramedullary hematopoiesis; and spontaneous transformation to acute leukemia⁷ or the development of marrow fibrosis but at a low rate compared with the rate in chronic myelogenous leukemia (CML).

This classification scheme, of course, implies that we know more about these disorders than we actually do. In fact, among hematologic disorders—particularly malignant ones—PV, IMF, and ET are among the least well understood and most understudied. For example, we do not know which multipotent hematopoietic stem cell is involved in these disorders, the significance of clonality or the lack of it, whether the disorders are interrelated to any extent, or how to reconcile their treatment with their clinical and clonal heterogeneity.

The recent World Health Organization classification of the chronic myeloproliferative diseases includes in addition to PV, IMF, and ET, the following disorders: CML (BCR/ABL positive), chronic neutrophilic

leukemia, chronic eosinophilic leukemia and the hypereosinophilic syndrome, and chronic myeloproliferative disease-unclassifiable,⁸ but only PV, IMF, and ET will be considered here.

There are many reasons for this unsatisfactory situation. First, while not rare, the chronic myeloproliferative disorders are sufficiently uncommon that most physicians, including hematologists, encounter very few such patients in the course of their practice. Second, these disorders are not only chronic, but their clinical manifestations also vary during the course of the illness, making it difficult for any one physician to observe their full clinical scope. Third, the chronic myeloproliferative disorders exhibit significant phenotypic mimicry, not only with respect to other benign and malignant blood diseases but also, and most important, among themselves. As a consequence, since molecular markers for these disorders are lacking, attempts to distinguish them on clinical grounds can be misleading. For example, approximately 10% of patients in one study of IMF actually had PV,⁹ while many of the patients initially described as having ET had PV instead.¹⁰ Indeed, PV can present as isolated leukocytosis,¹¹ isolated thrombocytosis,¹² or myelofibrosis with myeloid metaplasia,¹³ while IMF can also present with isolated thrombocytosis. Fourth, ironically, because the chronic myeloproliferative disorders were among the earliest recognized hematologic diseases, it is commonly perceived that these diseases have been well characterized, when in fact the bulk of clinical information about them is not evidence based but both anecdotal and distorted because of the confounding issues cited above that are intrinsic to these disorders. For example, the clinical phenotype for PV was derived from 13 case studies selected from a group of 75 patients,¹⁴ while ET was initially designated “hemorrhagic thrombocythemia”¹⁰ although current clinical concerns about this disorder focus on its thrombotic rather than its hemorrhagic manifestations.¹⁵ Fifth, investigator bias has contributed substantially to our lack of knowledge or confusion about the optimal treatment of the chronic myeloproliferative disorders. For example, the perceived efficacy

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of ^{32}P combined with lack of appreciation of the pathophysiology and natural history of PV led to the initial adoption of ^{32}P as the treatment of choice for this disease without the type of clinical trials that would be mandatory today and despite caveats about its toxicity.^{16,17,18} Even when a major prospective, controlled clinical trial involving PV was conducted,⁷ prejudice against phlebotomy therapy,¹⁹ inattention to gender, and the assumption that PV was a monolithic disorder led to inadequate trial design and, consequently, misleading results and conclusions.

Finally, from a basic science perspective, until recently, focus on the growth factor sensitivity of committed hematopoietic progenitor cells has overshadowed scrutiny of the actual target cell involved in the chronic myeloproliferative disorders, a multipotent hematopoietic progenitor cell, while the full complement of the tools of molecular biology has yet to be applied to the study of these disorders. Indeed, Osler's cogent 1908 comment about PV, "Nothing is more certain in the microcosm as in the macrocosm, given a demand there is soon a supply. But here is a condition in which, as far as we know, there is an over-supply without any corresponding demand,"²⁰ is still relevant, and its contention is supported by the current lack of consensus among hematologists about the appropriate means for diagnosis and the optimal therapy for this disorder.²¹ This chapter will focus on some topics of clinical controversy and areas of need with respect to these disorders.

II. INDICATIONS FOR CYTOREDUCTIVE THERAPY IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

Tiziano Barbui, MD, and Guido Finazzi, MD*

PV and ET are chronic myeloproliferative diseases (MPDs) in which there are a thrombohemorrhagic diathesis and a variable incidence of progression to myelofibrosis with myeloid metaplasia (MMM) or acute myeloid leukemia (AML). The main cause of mortality and morbidity is thrombosis, which occurs more frequently in older patients or in those with previous vascular complications. Severe bleeding is rare and limited to patients with a very high platelet count and to those taking antiplatelet drugs. Cytoreductive therapy is effective in preventing thrombosis, but there is concern that some myelosuppressive agents may increase the rate of hematological transformation. At present,

apart from anecdotal reports of bone marrow transplantation, there is no known treatment that eradicates the abnormal clone. Thus, the objective of therapy is twofold: first, to minimize the risk of thrombotic complications, and second, to prevent progression to myelofibrosis and acute leukemia.

Polycythemia Vera

History of treatments

Therapeutic recommendations in PV come from a very limited number of randomized clinical trials and a series of prospective and retrospective studies that have described the natural history of PV patients and indirectly evaluated the effect of different strategies on the main outcomes.

In the first study by the Polycythemia Vera Study Group (PVSG-01 trial),¹ 431 patients were randomized to one of the following treatments: (1) phlebotomy alone; (2) ^{32}P plus phlebotomy, and (3) chlorambucil plus phlebotomy. Patients treated with phlebotomy alone had a better median survival time (13.9 yrs) than those receiving ^{32}P (11.8 yrs) or chlorambucil (8.9 yrs). Causes of death were different in the 3 groups. Phlebotomized patients showed an excess of mortality within the first 2 to 4 years, principally caused by thrombotic complications. Those in the 2 myelosuppression arms suffered higher rates of acute leukemia and other malignancies developing later during the follow-up. The incidence of MMM was virtually identical in the 3 arms. In the late 1970s, the search for a nonmutagenic myelosuppressive agent led the PVSG to investigate hydroxyurea (HU), an antimetabolite that prevents DNA synthesis by inhibiting the enzyme ribonucleoside reductase. At that time, it was assumed that this agent would not be leukemogenic or carcinogenic. In the last PVSG report, 51 PV patients treated with HU were followed for a median and maximum of 8.6 and 15.3 years, respectively.² The incidence of acute leukemia, myelofibrosis, and death were compared with the incidence in 134 patients treated only with phlebotomy in the PVSG-01 protocol. There were no significant differences in any of the 3 parameters, although the HU group showed a tendency to more acute leukemias (9.8% vs 3.7%), less myelofibrosis (7.8% vs 12.7%), and fewer total deaths (39.2% vs 55.2%).

The efficacy and safety of HU in PV were also analyzed in a randomized clinical trial in France,³ in which 292 patients below the age of 65 were randomized to treatment with HU or pipobroman and followed from 1980 until 1997. Pipobroman is a bromide derivative of piperazine with a chemical formula similar to the alkylating agents, but its mechanism of action also in-

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volves metabolic competition with pyrimidine bases. No significant differences between the 2 groups were observed in overall survival, rate of thrombotic complications, and incidence of secondary leukemia (about 5% at the 10th year and 10% at the 13th year). There was a significant increase in the risk of progression to myelofibrosis in the patients treated with HU (26 cases) compared with those treated with pipobroman (3 cases). Busulfan was tested in a randomized clinical trial carried out by the EORTC:⁴ 293 PV patients were randomized to busulfan or ³²P and followed for a median of 8 years. Ten-year survival was significantly better in the busulfan group (70% vs 55%) because of a lower incidence of vascular deaths. There was no difference in the rates of MMM, AML, and cancer.

Based on these studies, PVSG produced the following recommendations that were shared by experts in the field.¹ Phlebotomy was suggested in all patients to keep the hematocrit below 0.45. Stable patients at low risk for thrombosis (age < 60 years, no history of thrombosis) might not require additional therapy. In patients at high risk of thrombosis or with a very high phlebotomy requirement, the choice of myelosuppressive agent was age-adapted. Older patients could be managed with ³²P, busulfan, or pipobroman; whereas HU was considered the agent of choice in younger patients.

PV care in the community

The impact of the PVSG studies on the routine management of PV by hematologists and oncologists in the United States has been recently assessed.⁵ A random sample of 1006 US American Society of Hematology (ASH) members was surveyed; the results indicated significant differences in the approach to the diagnosis and treatment of PV. Regarding the treatment of erythrocytosis, 69% of physicians used phlebotomy as their first choice, supplemented by HU in only 28% of cases. Therapy for thrombocytosis was given by 82% of physicians only when platelet counts exceeded $1000 \times 10^9/L$ or in the event of related symptoms. HU (63%) or anagrelide (35%) were the primary agents used.

The approach generally taken in Europe is equally heterogeneous. We have recently completed an epidemiological prospective study of 1638 PV patients (942 males and 696 females, median age 60.4 years at diagnosis and 65.4 years at recruitment) enrolled in 12 European countries (ECLAP study, unpublished data). The percentages of patients treated with different therapies in different countries varied widely. The use of phlebotomy alone ranged from 47% to 77%, HU from 43% to 75%, and ³²P from 0% to 11%. This reflects the uncertainty in the management of PV despite the PVSG recommendations.

Clinical Epidemiology of Polycythemia Vera

Incidence of thrombosis and hematological transformation

The current epidemiology of PV should be evaluated in large, prospective clinical studies with well-defined end points and external validation of events, as usually required for randomized clinical trials. This is the case of the ECLAP study, mentioned above. Of the 1638 patients enrolled, 633 (38.4%) had a history of thrombotic events. Respectively, about three quarters and one quarter of previous thromboses were arterial and venous and, among the former, ischemic stroke and transient ischemic attacks accounted for two thirds of events. The mean duration of follow-up was 2.7 ± 1.3 years (median 2.8 years; range 0–5.3 years), and overall mortality was 3.5 deaths/100 persons per year. During follow-up, nonfatal major thromboses were observed in 122 patients (7.4%), of which 87 were arterial (53 cerebral ischemia, 14 acute myocardial infarction and 20 peripheral arterial thrombosis) and 50 (3%), venous. Causes of mortality are reported in **Table 1**. Cardiovascular events and hematological transformation (mainly AML) were responsible for 41% and 13% of deaths, respectively; major bleeding accounted for only 4%. Progression to MMM occurred in 38 patients (2.3%), with an incidence rate of approximately 1% per patient-year, and the frequency rose steadily during follow-up.

Risk factors

In the ECLAP study, the incidence of cardiovascular complications was much higher in patients aged more than 60 years (hazard ratio 8.6, 95% confidence inter-

Table 1. Causes of death in 1638 patients with polycythemia vera in the European collaboration on low-dose aspirin in polycythemia vera (ECLAP) study.

All-cause mortality	164 (10%)
Fatal thrombosis	67 (4.1%)
Coronary heart disease	25 (1.5%)
Congestive heart failure	13 (0.8%)
Ischemic stroke	13 (0.8%)
Pulmonary embolism	6 (0.4%)
Other vascular death	10 (0.6%)
Fatal bleeding	7 (0.4%)
Cancer	54 (3.3%)
Acute leukemia	21 (1.2%)
Myelofibrosis	1 (0.1%)
Other cancers	32 (2.0%)
Other or unknown causes	36 (2.2%)

val [CI] 3.0-22.7, $P < .0001$) or with a history of thrombosis (hazard ratio 4.85, 95% CI 1.46-16.1, $P = .0099$) than in younger subjects with no history of thrombosis. Patients with both a history of thrombosis and age more than 60 years had the highest risk of cardiovascular events during follow-up (hazard ratio 17.3, 95% CI 6.4-47, $P < .0001$). These data confirm that increasing age and a history of thrombosis are the 2 most important prognostic factors for development of thrombotic events in patients with polycythemia.^{1,6} Other significant predictors of survival and cardiovascular morbidity were smoking habit, diabetes mellitus, and congestive heart failure.

Thus, there appears to be a group of PV patients at “intermediate risk” of thrombosis, whose existence has been suggested but never formally proved. In summary, PV patients can be stratified into 3 risk categories on the basis of their probability of developing thrombotic complications (**Table 2**). This classification forms the rationale for the indications to cytoreductive therapy.

Predictors of hematological transformation in the 1638 patients in the ECLAP study have also been analyzed. In a multivariate model, the main risk factor was the duration of disease. The relative risk of MMM and/or AML ranged from 1.4 to 6.3, respectively, after 3 and more than 10 years from diagnosis of PV. However, the relation between disease progression and duration, and therefore long-term cytoreductive therapy, means it is impossible to distinguish between the amount of risk of hematological transformation due to the natural course of disease and that due to aggressive, long-term use of myelosuppressive drugs.

New Treatments

Interferon- α

Interferon (IFN)- α suppresses the proliferation of hematopoietic progenitors, has a direct inhibiting effect on bone marrow fibroblast progenitor cells, and antagonizes the action of platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β), and other cytokines, which may be involved in the development of myelofibrosis.^{7,8} Most important, IFN- α is not known to be leukemogenic or teratogenic. To date, no controlled clinical trials have been published on its efficacy and safety in PV. Published reports concern small consecutive series of patients in whom hematological response and side effects were evaluated. One review analyzed the cumulative experience with IFN- α therapy in 279 patients from 16 studies.⁹ Overall responses were 50% for reduction of hematocrit to less than 0.45% without concomitant phlebotomies, and 77% for reduction in spleen size. Results from single-institution studies with long-term follow-up were similar.^{10,11}

The main problem with IFN- α therapy, apart from its costs and parental route of administration, is the incidence of side effects. Fever and flu-like symptoms are experienced by most patients and usually require treatment with paracetamol. Signs of chronic IFN- α toxicity, such as weakness, myalgia, weight and hair loss, severe depression, and gastrointestinal and cardiovascular symptoms, make it necessary to discontinue the drug in about one third of patients. Overall, the role of IFN- α in PV therapy requires further study, but controlled clinical trials evaluating long-term clinical end points are needed. The development of peg-IFN (polyethylene glycol formulation) may be one step forward, because of its slower clearance, permitting once-weekly dosing and perhaps fewer side effects.¹²

Anagrelide

Anagrelide is a member of the imidazo(2,1-b)quinazolin-2-one series of compounds, with inhibitory activity on platelet aggregation in humans and animals. In humans anagrelide also has a species-specific platelet-lowering effect at doses lower than those required to inhibit platelet aggregation. Because of this effect, the drug has been tested in patients with clonal thrombocytosis and has been shown to have potent platelet-reducing activity. The mechanism by which anagrelide reduces platelet count and decreases red blood cells to some degree as well without affecting the white count is not completely understood, but its main action is inhibition of megakaryocyte maturation.¹³ No chromosomal damage has been reported in relation to its use. The drug's efficacy has been assessed in nonrandomized clinical studies, mainly in essential thrombocythemia (ET) (see below). In PV, anagrelide has been used for reducing thrombocytosis on the assumption that lowering platelet counts may reduce the rate of thrombosis. However, this rationale is uncertain because no study has ever demonstrated a clear relation between platelet number or function and major vessel thrombosis.^{14,15} Therefore, this drug's

Table 2. Risk stratification in polycythemia vera (PV) and essential thrombocythemia based on thrombotic risk.*

Risk Category	Age > 60 years or History of Thrombosis	Cardiovascular Risk Factors
Low	no	no
Intermediate	no	yes
High	yes	

*Cardiovascular risk factors include hypertension, hypercholesterolemia, diabetes, smoking, and congestive heart failure. Extreme thrombocytosis (platelet count $> 1500 \times 10^9/L$) is a risk factor for bleeding. Its role as a risk factor for thrombosis in PV is uncertain.

final place in the therapeutic strategy of PV patients remains to be established in controlled clinical trials.

Experimental therapies

Allogeneic bone marrow transplantation (BMT) has been used in a few selected patients with PV with the aim of eradicating the malignant clone.^{16,17} Preliminary clinical experience indicates that some patients can be cured, while autologous stem cell transplantation has only a palliative effect.¹⁸ Patients who may benefit from allogeneic bone marrow transplantation are those who develop PV at a very young age or who rapidly progress to intractable MMM. However, it is too early to define the role of either allogeneic or autologous BMT in PV, and further studies are required, including improvements of nonmyeloablative techniques.

Some PV patients may respond to imatinib mesylate. Two patients with *bcr/abl*-negative PV have been recently reported; both were unable to tolerate either HU or IFN- α but had an excellent clinical response to imatinib mesylate.¹⁹ This positive result provides a new approach for selected PV patients that is worth further evaluation; however, at present, no controlled studies have been published and imatinib's role in therapy of PV remains anecdotal.

Recommendations for Cytoreductive Therapy

Although much progress has been made in updating the clinical epidemiology of PV and identifying new treatment options, current recommendations for cytoreductive therapy are still largely similar to those at the end of the PVSG era (**Figure 1**). Phlebotomy to reduce the red cell mass and keep it at a safe level (hematocrit < 45%) remains the cornerstone of treatment. Venesection is an effective and safe therapy and previous concerns about potential side effects, including severe iron deficiency and an increased tendency to thrombosis or MMM, have been recently overcome as we have gained a better understanding of the pathophysiology of the disease.^{14,15} Many patients require no other therapy for many years. For oth-

ers, however, poor compliance to phlebotomy or progressive myeloproliferation, as indicated by increasing splenomegaly or very high leukocyte or platelet counts, may call for the introduction of cytoreductive drugs.

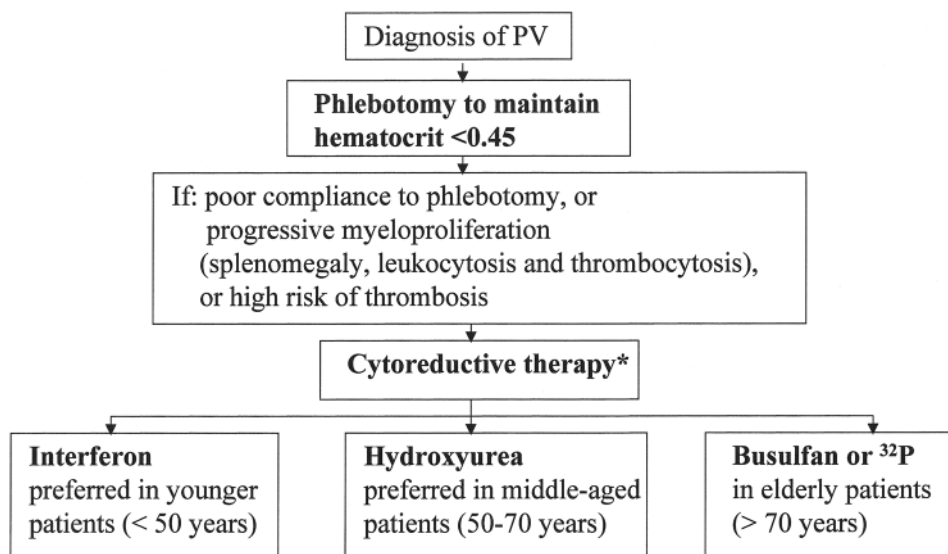
HU is the most frequently used cytoreductive agent, although doubts persist about its long-term leukemogenicity. IFN- α is a promising alternative, but it is expensive and its side-effects can be debilitating, particularly in the elderly. Thus, a reasonable approach could be to reserve IFN- α for younger patients (< 50 years) and recommend HU for the others. Busulfan and ³²P may have a role in patients over 70 years of age, and anagrelide can be considered to control extreme thrombocytosis (> 1500 \times 10⁹/L), particularly in patients with hemorrhagic or microvascular symptoms.

Essential Thrombocythemia

Survival and incidence of major thrombotic and hemorrhagic events

Five cohort studies compared overall survival in ET patients with that of the general population (reviewed in Barbui et al²⁰). Four of them (2 with statistical significance) found a lower life expectancy in ET patients than in age- and sex-matched controls. However, when the analysis was restricted to patients younger than 50 years, overall survival was similar to that of an age- and sex-matched control population.²¹

The incidence of thrombotic and hemorrhagic complications was analyzed in 1850 patients with ET from 21 retrospective cohort studies (reviewed in Barbui et



* Consider **Anagrelide** for control of symptomatic thrombocytosis

Figure 1. An algorithm of treatment recommendations in patients with polycythemia vera (PV).

al²⁰). The overall rates of thrombosis and hemorrhage at diagnosis ranged from 9% to 84%, and from 3.9% to 63%, respectively. After diagnosis, rates for thrombosis and hemorrhage during follow-up ranged from 7% to 17% and 8% to 14%. In our case-control study,²² the overall risk of thrombotic episodes was 6.6% per patient-year in a historical cohort of 100 patients with ET and 1.2% per patient-year in 200 patients with a benign monoclonal gammopathy of undetermined significance who served as controls. The rate of major hemorrhagic complications was 0.33% per patient-year. However, these various studies covered a wide range of patient populations, of definitions of major and minor vascular events, and of clinical settings for patient recruitment (hematology, vascular, thrombosis, and internal medicine units), so there was a high risk of both selection and referral biases.

Risk factors for thrombosis and bleeding

Age, a previous thrombotic event, and long duration of thrombocytosis were identified as major risk factors for thrombosis in the controlled series we reported.²² Age over 60 and history of major ischemic events were also risk factors for atherothrombotic complications in a large uncontrolled study of 148 ET patients.²³ A history of thrombosis at diagnosis was significantly associated with recurrent thrombosis, and a platelet count higher than $1500 \times 10^9/L$ at diagnosis was significantly associated with gastrointestinal tract bleeding in a series of young women.²¹

Recent studies have suggested that vascular complications can also be predicted from: (1) the biological characteristics of the disease, (2) markers of hypercoagulability, and (3) general cardiovascular risk factors. In particular, ET patients with clonal disease,²⁴ with impaired expression of *c-mpl* in bone marrow megakaryocytes,²⁵ or with overexpression of PRV-1 (Pahl HL, personal communication) were at higher risk for vascular complications.

Concerning testing for thrombophilia, factor V Leiden²⁶ and antiphospholipid antibodies²⁷ have been associated with an increased thrombotic risk in ET and PV patients. Among cardiovascular risk factors, hypertension,²³ hypercholesterolemia,²³ and smoking²⁸ were also associated with an increased risk of developing major vascular complications in some, but not all studies (**Table 2**).

Paradoxically, a very high platelet count ($> 1500 \times 10^9/L$) was a major predictor of bleeding rather than thrombotic complications.²⁹ An investigation of platelet count and large von Willebrand factor (VWF) multimers in the plasma of 36 patients with ET and 26 patients with reactive thrombocytosis, found an inverse relationship between

these parameters in both groups. Normalization of the platelet count was accompanied by restoration of a normal plasma VWF multimeric distribution.³⁰

Progression of the disease

ET may transform to myelofibrosis or AML as part of the natural history. In a series of 195 patients followed for a median of 7.2 years (range, 1.9–24), conversion to MMM was observed in 13 cases, with an actuarial probability of 2.7% at 5 years, 8.3% at 10 years, and 15.3% at 15 years.³¹ There are sporadic reports of the progression of ET to AML and other hematological malignancies, although the incidence appears less than in the related myeloproliferative diseases (MPD).³² Retrospective studies with a median follow-up of 3–7 years have reported rates of leukemic conversion ranging from 0.6% to 5%, but most of the patients involved had already received cytoreductive therapy.³³ In a series of 2316 patients retrospectively collected in Italy,³⁴ the rate of transformation into AML or myelodysplasia (MDS) was about 1% in patients left untreated. Use of IFN or HU alone gave a similar incidence of AML-MDS, whereas 4% of patients given alkylating agents transformed.³⁵

When to Start Platelet-Lowering Therapy

Avoiding cytoreduction is an option for low-risk ET patients. A natural history of these patients left untreated was prospectively evaluated in a controlled study³⁶ that compared 65 patients fulfilling the criteria for low-risk for thrombosis and 65 age- and sex-matched normal controls. Patients were followed up and cytoreductive therapy was introduced as soon as a major clinical event was recorded. After a median follow-up of 4.1 years, the incidence of thrombosis was not significantly higher in patients than in controls (1.91% vs 1.5% per patient-year; age- and sex-adjusted risk ratio 1.43, 95% CI 0.37–5.4). No major bleeding was observed. These findings were confirmed in another Italian cohort of 28 patients below 40 years of age who were followed for a median of 4.6 years,³⁷ and in 74 young women followed for more than 9 years, reported by Tefferi et al.²¹ In these 2 groups of untreated patients, the risk of recurrent thrombosis was 0.8 and 1.2 cases/100 patient-years, respectively.

Thrombotic deaths seem very rare in low-risk ET subjects, and there are no data indicating that fatalities can be prevented by starting cytoreductive drugs early. Therefore, withholding chemotherapy might be justifiable in young, asymptomatic ET patients with a platelet count below $1,500,000/mm^3$ and with no additional risk factors for thrombosis. This policy is based on the low risk of complications and the potential leukemogenicity of cytotoxic drugs. However, the strength of

these recommendations is based on studies with relatively small samples, and further data from large clinical trials are needed. If cardiovascular risk factors together with ET are identified (smoking, obesity, hypertension, hyperlipidemia) it is wise to consider platelet-lowering agents on an individual basis.

Which Platelet-Lowering Agent to Use

Hydroxyurea

Hydroxyurea (HU) has emerged as the treatment of choice in high-risk patients with ET because of its efficacy and rare acute toxicity. Its efficacy in preventing thrombosis in high-risk ET patients was demonstrated in a randomized clinical trial in which 114 patients (35 males and 79 females, median age 68 years, range 40–85 years; median platelet count $788 \times 10^9/L$, range 533–1,240,000/ mm^3) were randomized to long-term treatment with HU ($n = 56$) or no cytoreductive treatment ($n = 58$). During a median follow-up of 27 months, 2 thromboses (1 stroke and 1 myocardial infarction) were recorded in the HU-treated group (1.6%/pt-yr) compared with 14 in the control group (1 stroke, 5 transient ischemic attacks, 5 peripheral arterial occlusions, 1 deep vein thrombosis, and 2 patients with superficial thrombophlebitis) (10.7%/pt-yr; $P = .003$).^{38,39}

Hematopoietic impairment, leading to neutropenia and macrocytic anemia, is the main short-term toxic effect of HU. Other less frequent side effects include oral and leg ulcers and skin lesions. The leukemogenicity of this agent is still debated. Some long-term studies found that a proportion of ET patients treated with HU developed acute leukemia.⁴⁰ In other studies, however, this drug was rarely associated with secondary malignancies. In the database of Italian ET patients mentioned above,³⁴ the rate of transformation in those given HU as a single agent was not significantly different from that in untreated cases.³⁵ In a recent analysis of 25 ET patients younger than 50 years and treated with HU alone for a high risk of thrombosis, no leukemic or neoplastic transformation occurred after a median follow-up of 8 years (range 5–14).⁴¹

The incidence of acute leukemic transformation is higher in patients with ET who have cytogenetic abnormalities⁴² or are receiving multiple cytotoxic drugs with different mechanisms of action.^{39,40,43} The 17p deletion has been described in a high proportion of ET patients who developed AML and MDS after treatment with HU.⁴⁰ However, in vivo exposure to HU was not associated with any increase in the number of DNA mutations.⁴⁴

Many studies have looked into the association between multiple myelosuppressive therapies and the oc-

currence of AML/MDS in ET. In a long-term study of 112 patients, we observed that none of 20 patients never treated with chemotherapy developed neoplasia, as compared with 3 of 77 given HU only (3.9% n.s.) and 5 of 15 given busulfan plus HU (33%, $P < .0001$).³⁹ Sterkers et al reported 14% of leukemia when HU was combined with other cytotoxic agents, generally pipobroman.⁴⁰ Six cases of AML (21%) in 28 ET patients treated with HU plus alkylating agents or ³²P were observed by Murphy et al.⁴³

In the risk/benefit assessment, HU remains the first-choice drug for most ET patients requiring cytoreductive therapy because: (1) it is the only treatment proved to be effective in reducing thrombotic complications in a randomized clinical trial and (2) its leukemogenicity when used alone is very low, possibly nil. However, this drug should be considered with caution in younger patients and in those previously treated with other cytotoxic agents.

Anagrelide

Anagrelide is effective for reducing high platelet counts in a variety of chronic myeloid disorders and particularly in ET.⁴⁵ The largest study reported so far comprised 1618 patients (934 with ET) evaluated for efficacy and 3660 patients (2251 with ET) evaluated for safety.⁴⁶ A dose of 2.1–2.2 mg/day reduced platelets to less than $600 \times 10^9/L$ or by more than 50% from baseline in 67% of ET patients. With a maximum follow-up of 7 years, anagrelide did not increase conversion to AML.⁴⁶ The most serious complications of the drug are cardiac, including palpitations or forceful heart beats (27% of patients), tachycardia and other arrhythmias (< 10%), and congestive heart failure (2%).⁴⁵ No data from randomized trials are yet available, and it must be borne in mind that reducing platelet counts is a surrogate end point of efficacy whose association with clinical end points, such as major vascular complications, is uncertain.

This caveat is further underlined by the results of a recent study on the long-term efficacy and safety of anagrelide, in which clinical end points were carefully assessed⁴⁷; a series of 37 consecutive young patients with ET (median age 40 yrs, range 18–49 yrs) were followed in a single institution for a median follow-up of 10.7 years (range 5.2–13.7 yrs).⁴⁶ Platelet count was reduced to $< 600 \times 10^9/L$ in more than 80% of patients given the drug. However, 20% of the patients had thrombotic complications, and a similar proportion showed major hemorrhagic events. All thrombohemorrhagic complications occurred at a platelet count of more than $400 \times 10^9/L$, so quite possibly complete normalization of thrombocytosis is needed to minimize the incidence of recurrent bleeding and thrombosis. Eight patients

(24%) experienced a more than 3g/dL decrease in hemoglobin and none of them developed acute leukemia. In summary, anagrelide is fast acquiring the role of first-line agent for the treatment of thrombocytosis in young patients with MPD because of its efficacy in reducing platelet count without leukemic transformation. However, a formal demonstration of its efficacy in avoiding thrombohemorrhagic complications is still lacking, and the cost and side effects of the drug must be borne in mind. The benefits of anagrelide compared with conventional treatment (e.g., HU) need to be investigated in controlled clinical trials, such as those currently in progress in the UK (PT-1 study).

Interferon

IFN- α has been evaluated in several cohorts of ET patients (reviewed in Lengfelder⁴⁸). Platelet count was reduced to below 600,000/mm³ in about 90% of cases after about 3 months, with an average dose of 3 million international units (IU) daily. The time and degree of platelet reduction during the induction phase were dose-dependent. The IFN- α dose can be tapered during maintenance, but if it is suspended, the platelet count rebounds in the majority of patients. IFN- α is not known to be teratogenic and does not cross the placenta. Thus, it has been used successfully throughout pregnancy in some ET patients with no adverse fetal or maternal effects.

Side effects are a major problem with this drug, as mentioned earlier. In a series of 273 ET patients,⁴⁸ IFN- α therapy was terminated in 25% (67 cases) before completion of the primary treatment plan. The rate of withdrawal ranged between 0% and 66% in the different studies. This wide range may be partly explained by the difference in observation times, which went from 1 month to 4 years. The most common reasons for withdrawal were IFN- α -related side effects, seen in 55%, and patient refusal in 10%. So far, no leukemogenic effects have been reported. However, in a retrospective analysis of more than 2000 ET patients from our registry, 159 were given only IFN- α and 2 developed leukemia. It remains to be seen whether this was due to the inherent risk of ET to progress to AML. Despite its high cost and toxicity, IFN remains a promising agent in cytoreductive treatment of ET, especially in younger patients.

Pregnancy

The management of ET in pregnancy has to consider the potential risks to the mother and fetus. Only limited information is available so far, mainly from small

retrospective studies (reviewed in Griesshammer⁴⁹). In this age group, we expect a 2% per year rate of major thrombotic events, and the figure may be higher in pregnancy. First-trimester miscarriage is reported in about 40% of ET (twice that in the control group), but later obstetric complications are infrequent. In a single-institutional study from the Mayo Clinic of 43 pregnancies in 20 patients, Wright and Tefferi were unable to substantiate the benefit of specific therapy during pregnancy or delivery.⁵⁰ Consequently, they do not recommend any prophylaxis in asymptomatic pregnant women with ET.

In our practice, the need for cytoreductive therapy is dictated by previous thrombohemorrhagic events, previous pregnancy complications, or platelet counts in excess of 1,500,000/mm³. HU is not indicated because of the possible teratogenic effect seen in experimental animals given high HU doses. However, for pregnant women with inadvertent exposure to HU during the first trimester, there is no clear indication for termination of pregnancy. IFN may be safer than anagrelide and is the drug of choice when platelet-lowering drugs are indicated. To reduce thrombosis in the placenta, low-dose aspirin (75-100 mg) is recommended and low-molecular-weight heparin may be considered in high-risk women with previous thrombosis and for second trimester loss.

Consensus-Based Recommendations

Consensus-based practice guidelines for the therapy of ET have been developed recently in Italy²⁰ (Figure 2). Convincing evidence is emerging that the treatment of ET patients should be based primarily on the expected risk of major thrombotic complications. Although the

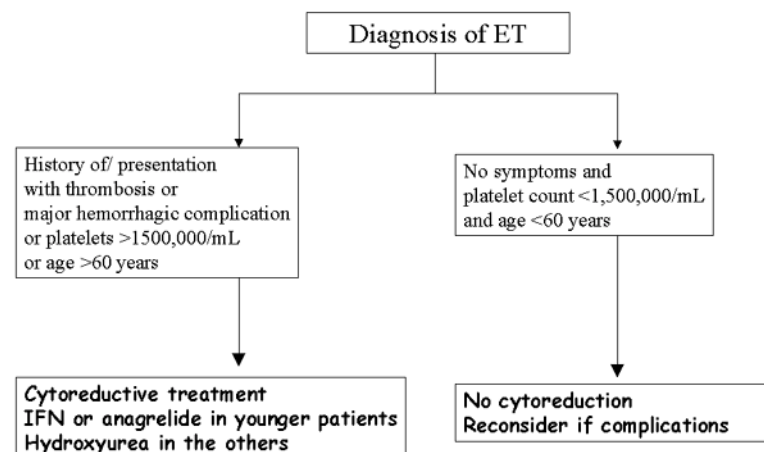


Figure 2. An algorithm of treatment recommendations in patients with essential thrombocythemia (ET).

Abbreviation: IFN, Interferon α .

specific values chosen for separating different risk categories are partly arbitrary, some recommendations can be made. Young asymptomatic subjects with platelet counts below $1500 \times 10^9/L$ are at lower risk and can be followed untreated. Any concomitant cardiovascular risk factors should be appropriately managed. Whether the presence of these adjunctive risk factors, which shift “low-risk” patients up into an “intermediate” class of risk, requires the introduction of cytoreductive therapy is still a matter of debate. For high-risk patients, HU (plus aspirin in the case of ischemia and/or thrombosis) is the treatment of choice because its efficacy in preventing thrombotic complications was demonstrated in a randomized clinical trial. However, the possible long-term leukemogenicity of this drug remains a major concern. Anagrelide and IFN- α could overcome this concern and should be considered for “high-risk” younger patients. However, their efficacy has been demonstrated only in lowering platelet counts, and clinical studies assessing clinical benefit are still needed.

Direction of Future Clinical Trials

In a field of rare diseases like MPDs, extensive networking of centers ready to share biological and clinical data is the necessary prerequisite for organizing future clinical studies. In addition to conventional end points, such as mortality, major thrombohemorrhagic events and hematological transformations, new biological markers of disease (PRV-1, clonality assays, and expression of *c-mpl* in bone marrow megakaryocytes) should be considered and validated as possible surrogate outcome measures. It is hoped that the combined effort of basic scientists and clinical investigators might improve the indications for cytoreductive therapy in PV and ET patients in the near future.

III. THE USE OF ASPIRIN IN POLYCYTHEMIA VERA

*Roberto Marchioli, MD, and Gianni Tognoni, MD**

Natural History of Polycythemia Vera

The clinical course in PV is marked by significant risk of thrombotic complications and a variable incidence of transformation into MMM and AML. Early studies in untreated patients found a high thrombotic incidence and a median survival of 18 months.

Tables 3 and 4 summarize the main epidemiologic and therapeutic trials in PV over the last two decades.¹⁻¹⁷ Clinical trials have assessed on one side the possibility

of lowering the risk of thrombotic events and on the other the risk of hematological transformations. As a quasi-rare disease, PV has posed a number of problems to researchers, and **Tables 3 and 4** could be read as didactical checklists of the methodological and operational problems still at the forefront of research and practice in the field of MPDs.

Methodological Issues

The “natural history” of the PV has been traced solely through the information collected in the context of research projects from selected groups of patients that are assumed to represent the entirety of the patient population. In particular, the burden of care and the clinical needs which are critically important for the adequate planning of trials aiming to assess the outcomes of treatment strategies are estimated on cohorts whose size hardly allows adequate stratification with respect to clinical and biological characteristics.¹⁻¹⁷

The disease is still elusive with respect to etiological and prognostic features. Thus, rigorous methodology should be utilized in order to bring about the advancement in knowledge and care.

The aforementioned methodological constraints are easy to recognize when examining the decision whether or not to treat PV patients with aspirin in an attempt to reduce the incidence of major thrombotic events. The Polycythemia Vera Study Group (PVSG) 05 trial (**Table 3**) was published in 1986 as the most authoritative reference in the field, and was designed to provide rigorous evidence of benefit as opposed to previous therapies adopted mostly by consensus.¹⁶ Its premature closure because of an excess of hemorrhagic events was controversial, and unfortunately did not lead to more intensive clinical research studies aimed to test better targeted therapeutic strategies. Indeed, it coincided with a moratorium of controlled clinical studies in PV in an era where in all fields of medicine the randomized clinical trial (RCT) had become the routine tool for efficient testing of new hypotheses.

The context for developing PV treatment strategies, and the use of aspirin in particular, should fall within the following research paradigms:

a) Outcome-oriented epidemiology. This should be a mandatory component of research activity of all those who care for rare diseases, to allow a reasonably documented profile of the disease natural history, through periodical reports.

b) Research networking. To be representative of the expected variability of practice, the philosophy of collaboration should be translated into permanent research networks aimed at sharing data according to predefined rules.¹⁸

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Table 3. Main epidemiological studies in polycythemia vera (PV).

Study	Design, No. of Patients, Follow-up	Treatments	Results, Comments
Brussamolino ¹	Cohort prospective study on 100 PV pts. Median follow-up 5 years	Pipobroman alone or in association	Median survival 140 months. Death rate at 12 years: 23%. Risk of AML at 5 years: 6%. Risk of AML at 7 years: 9%.
Najean ^{2,3}	Cohort prospective study on 175 PV pts. Follow-up 10 years.	Reduced dose of ³² P ± low-dose HU	Survival at 5 years: ³² P alone: 88%; ³² P + HU: 79% Survival at 7 years: ³² P alone: 77%; ³² P + HU: 66%
Lofvenberg ⁴	Cohort prospective study on 59 pts with PV, ET, myelofibrosis	HU	After 5 years survival (> 86%) similar to a sex- and age-matched Swedish population. Thrombotic event incidence: 20.8%.
Weinfeld ⁵	38 pts with PV, ET. Median follow-up > 10 years	HU	AML in 4/38 (10.5%)
Lofvenberg ⁶	81 PV and ET pts. Median follow-up 4 years	HU	AML in 4/65 (6.2%)
Najean ⁷	96 PV pts. Median follow-up 5.3 years. Pipobroman	HU	Absence of carcinogenic risk at short- but not necessarily at long-term
Messinezy ⁸	Retrospective study on 65 PV pts. 20 years follow-up	Venesection + low-dose busulfan	Median survival 11.1 years. O/E = 1.86 (<i>P</i> < .05). AML incidence 3.5%.
Rozman ⁹	Retrospective study on 1067 pts (PV 43%; ET 23%; M 34%). Median follow-up 4.3 years.	—	Observed survival not different from an age- and sex-matched Spanish population for PV and ET, but significantly worse for myelofibrosis. O/E in PV: 1.01; O/E in ET: 1.22; O/E in myelofibrosis: 1.59;
Brandt ¹⁰	Retrospective on 366 PV or ET pts. Median follow-up 7.2 years	³² P ³² P	162/366 deaths. O/E = .38. 17/366 cases of AML (4.6%), apparent accumulation of AML cases 8–12 years after start of treatment. Excess mortality 8–10 years after start of treatment
GISP ¹¹	Retrospective study on 1213 PV pts. Median follow-up 5.3 years	—	Death rate 2.94 per 100 PY. O/E = 1.69 (<i>P</i> < .05). Increase in risk of malignancies 6 years after diagnosis in pts receiving chemotherapy agents. Adjusted RR of death and major non-fatal thrombosis 2.1 (95%CI 1.4–3.1)

Abbreviations: ET, essential thrombocythemia, HU, hydroxyurea, AML, acute myeloid leukemia; O/E, observed events to the events expected in an age- and sex-matched reference population

c) Clinical practice as a normal research environment. The clinical epidemiology of practices and outcomes of PV (and other MPDs) should not be the occasional research object of outside experts. It must be the normal task of the daily caretakers of PV patients, as well as the reference framework of those who are working specifically on clinical and basic science research issues.

d) “Practice” of clinical trials. Both the formal testing through a RCT of reasonably documented hypotheses and the investigation of biological variables may be seen as expressions of an epidemiologically oriented clinical practice.

The Aspirin Hypothesis in PV

Clonal proliferation of myeloid precursors leads to progressive expansion of the red cell mass¹⁹ and results in blood hyperviscosity, the major determinant of circulatory disturbances and a common clinical manifestation of polycythemia vera.²⁰ Clinical observations made

in patients not receiving any cytoreductive treatment reported a very high incidence of vascular events, mainly in the cerebral circulation.²¹ Thrombotic complications in patients receiving cytoreductive treatment are far less frequent than in untreated patients but still remain a major cause of morbidity and mortality.¹¹ It is current clinical practice to use chemotherapy in addition to venesection in older patients and in those with previous vascular events.¹² The management of patients considered to be at lower risk of vascular occlusion is more controversial due to substantial uncertainty concerning the mutagenic risk of chemotherapeutic agents and the risk/benefit ratio of other treatments. Furthermore, current estimates of the individual vascular risk are largely empirical and are based on the combination of classical risk factors together with some disease-related parameters of uncertain significance such as platelet and leukocyte counts.

The efficacy and safety of antithrombotic drugs is

Table 4. Main trials in polycythemia vera (PV).

Trial	Patients	Endpoint	Experimental treatments			P
			32P	Phlebotomy	Chlorambucil	
PVSG-01 ¹²	431 pts; 18 yrs (max)	Median survival	11.8 yrs	13.9 yrs	8.9 yrs	0.02
		Thrombosis	30%	34%	25%	0.08
		Acute Leukemia	10%	1.5%	13%	< 0.0012
EORTC ¹³	293 pts. 8 yrs (median)	10-year survival	55%	70%		0.02
		Vascular deaths	18%	5%		n.r.
Najejan et al ¹⁴	461 pts; Age > 65 yrs 16 yrs (max)	Median survival	10.9 yrs	9.3 yrs.		n.s.
Najejan et al ¹⁵	292 pts; Age <65 yrs 16 yrs (max)	14-year survival	70%	70%		n.s.
		Myelofibrosis	17%	2.1%		0.03
PVSG-05 ¹⁶	166 pts; 1.2 yrs (median)	Thrombosis	ASA (900 mg/d)*	32P		n.r.
			8%	2%		
		Hemorrhage	7%	0%		0.02
GISP ¹⁷	112 pts; 1.4 yrs (median)	Thrombosis	ASA (40 mg/d)	Placebo		n.s.
			5%	7.7%		
		Hemorrhage	1.7%	1.9%		n.s.

Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; O/E, observed events to the events expected in an age- and sex-matched reference population; AML, acute myeloid leukemia; RR, relative risk; Pts, patients; HU, hydroxyurea; n.r., not reported; n.s., not significant; max, maximum follow-up; pts, patients.

*plus phlebotomy and dipyridamole

also unknown. Aspirin has long been avoided due to the results of a single study promoted by the Polycythemia Vera Study Group¹⁶ demonstrating an increased risk of bleeding and no reduction of thrombotic complications; however, a larger dose of ASA was used in this study than currently practised.

A pathophysiologic finding supporting the use of aspirin is the consistently increased in vivo thromboxane biosynthesis detected in polycythemia vera patients.¹⁸ This evidence generated the hypotheses that a) thromboxane-dependent platelet activation might be a major determinant of the increased thrombotic risk in polycythemia vera; and that b) low-dose aspirin might provide a rational and safe antithrombotic approach in these patients.

The GISP project and design

In 1993 the Gruppo Italiano di Studio sulla Policitemia Vera (GISP) decided to re-evaluate the natural history of PV,¹¹ as well as to launch a pilot trial to assess the feasibility of large-scale clinical trials in the field.¹⁷ Accordingly, a retrospective cohort study of 1213 PV subjects followed over 20 years in an ambulatory care

setting at 11 Italian hematological institutions was performed. The main outcome measures were all-cause mortality, venous plus arterial thrombosis, and hematological and non-hematological neoplastic disease. Six hundred thirty-four fatal and non-fatal arterial and venous thromboses were recorded in 485 patients (40%), 36% of the episodes being observed during follow-up in 230 patients (19%) and 64% occurring either at presentation or before diagnosis. Thrombotic events were more frequent in the two years preceding diagnosis, suggesting a causal relationship between the latent myeloproliferative disorder and the vascular accident. The incidence of thrombosis during follow-up was 3.4%/year, with evidence of higher risk in patients with advanced age and/or previous thrombotic history. Overall death rate was 2.9 per 100 patients/year, while thrombotic events and hematological or non-hematological malignancies had similar impact on mortality. Death from malignancy was three to four times more frequent in subjects receiving chemotherapy. The increase in risk of malignancies in patients treated with myelosuppressive agents became evident at the sixth year after diagnosis. In addition, the combined end-point

Table 5. Main results of the pilot trial on low-dose aspirin treatment conducted by the Gruppo Italiano Studio sulla Policitemia vera (GISP).

	Placebo (n = 52)	ASA (n = 60)
Non-fatal thrombosis	3	3
Peripheral arterial thrombosis	0	2
Transient ischemic attack	3	1
Non-fatal hemorrhages	1	1
Multiple hematomas	0	1
Gastrointestinal bleeding	1	0
Fatal events	2	0
Pulmonary embolism	1	0
Neoplasm of cholecyst	1	0

computed as the sum of the hardest available events (death or non-fatal myocardial infarction or stroke) suggested an overall unfavorable impact of myelo-suppressive agents.

The question of the utility of aspirin in PV was addressed in a GISP pilot clinical trial (Table 5). Over a nine-month period, 326 PV patients were screened at 8 hematological centers, and 112 (42 females and 70 males, aged 17 to 80) were found that did not have a clear indication for or contraindication to aspirin treatment. Patients were allocated by means of a double-blind, placebo-controlled, centrally-coordinated randomization to receive oral aspirin or placebo. Comparable risk profiles were obtained by randomizing according to age, disease duration, thrombotic history and current treatment. Follow-up duration was 16 ± 4 months. At the end of the study, 71 subjects (aspirin: 37, placebo: 34) were still on treatment. No difference was apparent as to hemorrhage, intolerance, and thrombotic events between the two groups. Irrespective of platelet count, measurements of serum thromboxane A2 production during whole blood clotting allowed demonstration of a complete inhibition of platelet PGG/II synthase activity in patients receiving aspirin.

The ECLAP project and design

The successful collaboration of GISP inspired a multicountry expansion, and offered the opportunity to utilize a prospective strategy. The flow-chart (Figure 3; see Appendix, page 618), which summarizes the conceptual and operational characteristics of the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) Project, reflects the feasibility and the implications of the general principles illustrated in the previous section. The ECLAP Study was a multi-country, multicenter project funded by the European Union which aimed to describe the natural history of poly-

cythemia vera as well as the efficacy of low-dose aspirin (Landolfi et al, unpublished data; Marchioli et al, unpublished data;^{22,23}).

The study design was based on the “uncertainty principle,” which reflects the “common sense” generally adopted in routine clinical practice in choosing between expectant and intervention management strategies. The scientifically and ethically appropriate answer to the condition of uncertainty is randomization. In other words, on the basis of clinical judgment, physicians categorized PV patients into three groups:

1. Subjects with clear benefit expected from the use of aspirin;
2. Subjects with clear contraindications for aspirin;
3. Subjects for whom clinicians are still substantially uncertain as to the benefit/risk ratio of aspirin therapy.

Only patients for whom clinicians were still substantially uncertain as to the benefit/risk ratio of aspirin therapy (last group) were randomized in the study since the recruitment of the other patients would be unethical. Non-randomized patients (along with causes of exclusion) were entered into a prospective observational study that was managed in parallel and used the same methodological rules of the clinical trial.

Overall, 1638 PV patients from 12 countries were included in the ECLAP Project. According to the uncertainty principle, 518 (32%) of these patients without a clear indication or contraindication to aspirin treatment were included in a parallel, double-blind, placebo-controlled, randomized clinical trial aimed at assessing the efficacy and safety of low-dose aspirin (100 mg daily) in addition to receiving standard cytoreductive therapy.

The two primary combined efficacy endpoints of the trial were the cumulative rate of non-fatal myocardial infarction, cardiovascular death or non-fatal stroke and the cumulative rate of non-fatal myocardial infarction, non-fatal stroke, pulmonary embolism, major vein thrombosis or cardiovascular death. The mean follow-up duration was about 3 years. All analyses were performed on an intention-to-treat basis.

The patients with PV enrolled in the study had an average age of 65 years (Table 6). Six hundred thirty-three patients (38.6%) had a history of thrombotic events. Arterial and venous thrombosis accounted for about 5% and 25% of previous thromboses, respectively. Ischemic stroke and transient ischemic attacks accounted for two-thirds of arterial thromboses, while deep vein thrombosis represented approximately 60% of venous thromboses. The incidence rates of cardiovascular and noncardiovascular mortality were 1.68 and 1.80 deaths/100 persons per year, respectively (Figure

Table 6. Baseline characteristics in 1638 patients with polycythemia vera recruited in the ECLAP Project.

Demographics	n
Age at recruitment (years)	65.4 (12.7)*
Males	942 (57.5)
Body mass index, kg/m ²	25.4 (3.7)*
Age at diagnosis (years)	60.4 (13.2)
Years from diagnosis to enrollment	
0–2	581 (35.5)
3–5	434 (26.5)
6–10	387 (23.6)
> 10	236 (14.4)
Prior cardiovascular events	
Prior thrombosis	633 (38.6)
Prior arterial thrombosis	470 (28.7)
AMI	146 (8.9)
Stroke	145 (8.9)
Transient ischemic attack	169 (10.3)
Peripheral	90 (5.5)
Prior venous thrombosis	225 (13.7)
Deep vein thrombosis	134 (8.4)
Pulmonary embolism	39 (2.4)
Superficial thrombophlebitis	100 (6.1)
Erythromelalgia	86 (5.3)
Intermittent claudication	77 (4.7)
Prior bleeding	133 (8.1)
Packed cell volume (l/l)	0.47 (0.06)*
Packed cell volume (l/l)	
≤ 0.45	556 (38.9)
0.46–0.50	530 (37.0)
> 0.50	345 (24.1)
Platelet count (×10 ⁹ /L)	398 (208)*
White blood cell count (×10 ⁹ /L)	10.9 (8.6)*
Antiplatelet drugs	955 (58.3)
Cytoreductive treatments	
Phlebotomy	1040 (63.5)
Any cytoreductive drug	1009 (61.6)
Hydroxyurea	793 (48.4)
Pipobroman	106 (6.5)
Interferon	64 (3.9)
Busulfan	61 (3.7)
³² P	44 (2.7)
Chlorambucil	5 (0.3)

* For continuous variables, values are mean ± SD.

4; see Appendix, page 618). Cardiovascular mortality and deaths from hematological transformations accounted for 45% and 13% of all deaths, respectively. Interestingly, almost 60% of patients were being treated with antiplatelet drugs because of their high cardiovascular risk already documented by their recurrent thrombotic events.

Results of the ECLAP trial

Baseline demographic and clinical characteristics were well balanced across the two groups. The main reasons for excluding the patients from the randomized trial were: need for antithrombotic therapy (66%), contraindication to aspirin (24%), and patients' unwillingness to participate (18%). Accordingly, a middle-aged population (61 years) of PV patients, with 26% of patients aged 70 years or older, 10% with prior thrombotic events, and 3% with prior hemorrhages, was randomized into the clinical trial. About 30% of patients had been diagnosed with PV in the year preceding the recruitment in the study. Only the percentage of current smokers and those using digitalis was not distributed evenly in the two arms. At baseline, 36% of the patients were managed by phlebotomy only, 21% by chemotherapeutic agents alone, and 36% by phlebotomy plus chemotherapeutic agents. Packed cell volume was kept at a median value of 0.46 during follow-up, with 25% of patients having levels higher than 0.48. Platelet count was kept at a median value of $321 \times 10^9/L$ during follow-up, with 25% of patients having levels higher than $460 \times 10^9/L$.

As to the results of the trial on the efficacy and safety of low-dose aspirin, the experimental treatment lowered the risk of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke (relative risk 0.41 [95% CI 0.15–1.15], $P = .0912$) and of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, pulmonary embolism, and major vein thrombosis (relative risk 0.40 [95% CI 0.18–0.91], $P = .0277$). Total and cardiovascular mortality were also reduced by 46% and 59%, respectively (Figure 5). Major bleedings were slightly increased nonsignificantly by aspirin (relative risk 1.62, 95% CI 0.27–9.71)

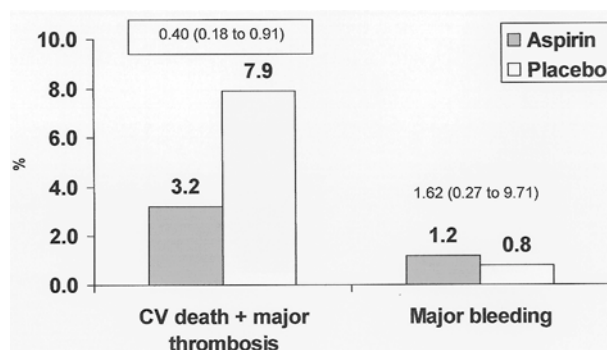


Figure 5. Risk/benefit profile of low-dose aspirin (100 mg daily) in 518 polycythemia vera patients enrolled in the ECLAP trial.

Clinical Implications

Consistent with the trial hypothesis and with the more general consensus on a mandatory, non-aggressive antiplatelet strategy in primary as well as in secondary prevention, low-dose aspirin should be considered as an evidence-based component of the long-term management of the PV population with the prognostic risk characteristics of the patients recruited in the ECLAP trial. Taken together with the information provided by the observational study, it could be assumed that for all PV patients (with the exception of those with a clear contraindication) an antithrombotic preventive strategy with low-dose aspirin is recommended.

The benefit/risk profile of aspirin in PV is in this sense comparable and consistent with the one that has imposed low-dose aspirin as one of the backbones of primary and secondary prevention in the area of cardiovascular risk.²⁴

The implications are not restricted to care. The adoption of aspirin as part of the routine treatment of PV modifies importantly the characteristics of the populations where other antithrombotic therapies are proposed and tested. The lowering of the thrombotic risk of PV imposes a more difficult challenge for agents aiming at an efficacy profile “superior to aspirin.” The safety profile is interesting not only as clinically essential information, but also as suggesting that the background pathophysiology of bleeding was not substantially modified in PV.

The ECLAP results have been obtained in settings of care and conditions of control that broadly mimic routine clinical practice. They could be considered representative therefore of what PV patients could experience under the care of competent practitioners. Seen against the broader cultural and methodological scenarios outlined in the first part of this chapter, the results of ECLAP have other implications as well. It has been shown that properly sized trials can be carried out (also with very limited financial support) in rare diseases like PV, over a relatively short period of time. The originally planned size of two thousand patients is realistically reachable in a network of fewer than 100 collaborating centers. The “happy ending of the (low-dose) aspirin story” leads to the beginning of a new approach for the control of the thrombotic risk of PV. While we hope that the findings presented here will be rapidly translated into practice, it is also important that practicing hematologists recognize the importance of working in large collaborative groups.

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IV. MYELOFIBROSIS WITH MYELOID METAPLASIA: AN UPDATE

Giovanni Barosi, MD, and Monia Marchetti, MD*

Pathogenesis

Current knowledge about the pathogenesis of MMM does not allow delineation of a single model that explains the proliferative advantage of the hematopoietic stem cells, the disruption of normal bone marrow extracellular texture with fibrosis, and extramedullary hematopoiesis. MMM has invariably been reported to be a clonal disorder involving erythroblasts, megakaryocytes, granulocytes, monocytes, and B and T lymphocytes,¹ documenting its origin from a pluripotent progenitor. Anomalies of expression of basic fibroblast growth factor (b-FGF) and its type I and type II receptors, and of transforming growth factor- β (TGF- β) and its receptors, are involved both in myelofibrosis and myeloproliferation.²

New concepts of the biology of the disease could shed light on the nature of cellular proliferation abnormalities in MMM and could explain the unique phenotype of the disease.

Abnormal CD34⁺ Cells Phenotype and Trafficking

The number of CD34⁺ progenitor cells determined by immunostaining in the bone marrow of patients with MMM appears to be increased in the early hypercellular stages indicating a higher proliferative activity of the precursor cell pool. When the disease evolves into an overt fibrosclerotic stage, bone marrow progenitor cells are usually reduced in number.³ At the same time, hematopoietic stem cells mobilize and migrate from the bone marrow to the bloodstream and colonize the spleen and other organs. The spleen content of hematopoietic CD34⁺ cells in MMM patients is increased from 2 to 10 times that of normal controls, and the concentration of spleen CD34⁺ hematopoietic progenitors is in tight equilibrium with the pool of circulating progenitors (G. Barosi et al, unpublished data). The spleen microenvironment is specifically appropriate for survival and proliferation of MMM hematopoietic progenitors. Spleen fibroblasts constitutively express and produce in culture a set of surface adhesion-related molecules, growth factors, and cytokines/chemokines relevant to normal steady state hematopoiesis.⁴ In particular, spleen MMM fibroblasts show a higher expression of ICAM-1 and VLA-4 than normal spleen fibroblasts.

Cell phenotype analysis has revealed that circulating CD34⁺ cells of MMM patients exhibit the antigenic profile of primitive progenitors, as evidenced by the presence of a high percentage of CD38^{low} and Thy-1⁺

cells,^{5,6} and express the c-kit multilineage receptor.⁵ Furthermore, when cultured in contact with spleen-derived fibroblasts isolated from MMM patients, CD34⁺ progenitors from patients with MMM produce myeloid cells in high numbers, while lymphoid cells are missing or markedly reduced in number because of an endogenous dysregulated IL-15 signaling pathway.⁵

Megakaryocyte Proliferation and Maturation Defects

In MMM, the proliferation of megakaryocytes has been repeatedly discussed in relation to the induction of an abnormal cytokine milieu that is critical for the synthetic stimulation of polyclonal fibroblasts, causing collagen fibrosis. Recent studies have reinforced the role of megakaryopoiesis in the pathogenesis and phenotype of the disease.

At the cellular level, the proliferative advantage of the megakaryocyte lineage is demonstrated by the elevated growth of hematopoietic progenitor cells *in vitro*, by their enhanced sensitivity to thrombopoietin (TPO), or by their autonomous growth. MMM platelets express a TPO-receptor (Mpl) isoform that is incompletely glycosylated and poorly expressed on the cell surface.⁷ The abnormal Mpl occurs at the stem cell level and is due to a defective posttranslational processing. Increased alternative splicing of Mpl produces a molecule defective in its distal cytokine binding domain, which is involved in its negative regulation. The TPO-receptor defect is not restricted to patients with MMM but also occurs in the majority of patients with PV, whose CD34⁺ cells, despite reduced Mpl expression, respond *in vitro* to TPO, linking abnormal Mpl expression with resistance to apoptosis and the aberrant signal transduction that provides a proliferative advantage. This abnormality also fits with the finding of elevated levels of serum TPO in patients with MMM.⁸

Experimental models in which mice, given bone marrow grafts of cells infected with a retrovirus carrying TPO complementary DNA, develop a lethal myeloproliferative disorder with several characteristic features of human MMM (TPO mice), reinforce the role of megakaryocyte proliferation in the pathogenesis of MMM.⁹⁻¹¹

Increased numbers of megakaryocytes in the marrow are necessary but not sufficient for the development of the disease in TPO mice. Wild-type and severe combined immunodeficient (SCID) mice that overexpress human TPO develop myelofibrosis, but nonobese diabetic (NOD)-SCID mice infected with the same virus do not.⁹ Because these mice all express similarly high levels of TPO in the serum and a high number of megakaryocytes in the marrow, genetic compo-

nents, different from those that regulate megakaryocyte cells, may be important cofactors in the development of the disease. Because NOD-SCID mice have also a monocyte defect, this suggests that monocytes in combination with megakaryocytes are required to induce myelofibrosis. The human counterpart of this observation is the documentation of a spontaneous activation of the nuclear factor- κ B (NF- κ B) pathway in monocytes of patients with MMM.¹⁰

The role of TGF- β in the promotion of myelofibrosis is documented by engrafting mutant TGF- β 1^{-/-} hematopoietic cells infected with a retrovirus encoding TPO into lethally irradiated wild-type hosts. While myelofibrosis is systematically produced in hosts repopulated with wild-type cells, no increase in reticulin deposition was seen in mice reconstituted with TGF- β 1^{-/-} donor cells, demonstrating that TGF- β 1 was essential for the promotion of myelofibrosis.¹¹

Moreover, TPO mice provide insight into one possible pathophysiologic explanation for the leakage of α -granular megakaryocyte contents into the bone marrow microenvironment that generates myelofibrosis. In the mouse model, a markedly abnormal subcellular distribution of P-selectin, a protein located in the α -granular membrane, appears to correlate with excessive and pathologic emperipoiesis of polymorphonuclear leukocytes in the megakaryocytes resulting in a release of α -granular proteins and growth factors.¹² The human counterpart of this observation is an increased emperipoiesis of polymorphonuclear cells by megakaryocytes of MMM.¹²

The possible role of osteoprotegerin (OPG) in the development of osteosclerosis is demonstrated when bone marrow stem cells from OPG knockout mice (OPG^{-/-}) infected with a retrovirus encoding the murine TPO are engrafted into lethally irradiated OPG^{-/-} or wild-type (WT) mice. Severe osteosclerosis is observed only when transplantation is performed in a WT background and correlates with a marked elevation of OPG in plasma and a decrease in the number of osteoclasts in the femur. In contrast, only rare bone trabeculae emerging from the cortical surface were seen in hosts lacking OPG in the microenvironment. OPG is a secreted molecule that binds RANKL and strongly inhibits osteogenesis by blunting the RANKL interaction and the RANK receptor (receptor activator of NF- κ B).¹³

The role of megakaryocytes in the development of myelofibrosis is demonstrated by mutant mice mutated GATA-1 (GATA-1^{low}), a transcription factor that exerts a well-established role in erythroid, megakaryocytic, and mast cell differentiation.^{14,15} These mice become anemic from 15 months on, and the anemia is associated with several markers of myelofibrosis such as tear-

drop poikilocytes and progenitor cells in the blood, collagen fibers in the marrow and in the spleen, and hematopoietic foci in the liver. The GATA-1^{low} mutation impairs the capacity of megakaryocytes to differentiate into platelets and growth factor genes implicated in the development of myelofibrosis (such as osteocalcin, TGF- β , platelet-derived growth factor [PDGF], and vascular endothelial growth factor [VEGF]) are all expressed at higher levels in the marrow from the mutants than normal mice.

Neoangiogenesis

Neoangiogenesis, or formation of new vessels, has emerged as one of the hallmarks of MMM among the chronic myeloproliferative disorders (CMDs). With a visual immunohistochemical microvessel grading method that uses a CD34 antibody, Mesa et al¹⁶ reported that approximately 70% of the patients with MMM had a substantial increase (grade 3 or 4) in bone marrow microvessel density compared with 33% of those with PV and 12% of those with ET. Furthermore, none of the patients with either PV or ET displayed grade 4 bone marrow angiogenesis, whereas 32.5% of the patients with MMM did.

Neoangiogenesis in MMM has now been documented as an integral component of medullary and extramedullary hematopoiesis, involving the major site of myeloid metaplasia, the spleen (G. Barosi et al, unpublished data). Spleen vasculature is a complex network of arterioles, capillaries, and sinuses, with different functions and different staining properties. By applying 2 different antibodies, anti-CD34 and anti-CD8, in spleen sections of MMM patients, both CD34⁺ endothelial cells lining small-caliber capillary vascular structures and CD8⁺ endothelial cells lining thin-walled and markedly ectatic sinuses are easily recognizable and are clearly distinguishable from the simultaneously stained CD34⁺ progenitor cells or CD8⁺ lymphocytes. In patients with MMM, the CD34⁺ capillary vascular density is from 2.15 to 4.2 times higher than in normal individuals, while sinusoidal vascular density is normal or lower than normal. Mesa et al¹⁶ documented a correlation between angiogenesis and bone marrow cellularity. Similarly, a correlation exists between increased capillary vascular density and the amount of spleen and blood CD34⁺ hemopoietic stem cells (G. Barosi et al, unpublished data).

A humoral mechanism for increased angiogenesis in MMM has been postulated. A recent study has demonstrated increased serum levels of VEGF in most patients with MMM,¹⁷ suggesting that the cytokine-mediated stromal reaction in MMM induces angiogenesis. However, we were able to measure, among the popula-

tion of spleen progenitors, a CD34⁺/AC133⁺/VEGFR2⁺ population of cells that have high potential for endothelial differentiation,¹⁸ namely, suggesting their possible role in promoting neovascularization in MMM diseased organs (G. Barosi et al, unpublished data).

Diagnostic Criteria

Setting the diagnostic criteria is a prerequisite in order to standardize the conduct and reporting of clinical studies and is of help for practitioners in clinical practice. Judging insufficient the set of criteria proposed by the Polycythemia Vera Study Group in 1976,¹⁹ 2 clinical research projects were launched recently. The first has originated from the strong hypothesis of a prefibrotic phase/type of the disease and includes megakaryocyte morphology, featured by the characteristic shape with plump lobulation of nuclei and asynchronous nuclear cytoplasm maturation, which is characteristic of MMM bone marrow biopsy. With the Cologne criteria, the authors (Thiele and coworkers²⁰ and Michiels and Thiele²¹) proposed a classification of MMM with diagnostic, staging, and prognostic value in which, besides presence of splenomegaly, the leuko-erythroblastic picture of peripheral blood, anemia, thrombocytosis, and clustering of megakaryocytes in bone marrow were used to define the diagnosis and the progression of the disease (**Table 7**). These criteria have been also accepted by the World Health Organization classification of MMM.

The second clinical research project was based on the notion that no causal/biological hypothesis currently identifies the principal parameters for the diagnostic definition of MMM, and that only a consensual, nominalistic approach should be possible. The Italian Cooperative Group on Myeloproliferative Disorders²² developed a definition of MMM using the literature-derived evidence on sensitivity and specificity of a core set of diagnostic criteria and the consensus methodology (**Table 8**).

In the absence of biological markers for the specific diagnosis of the disease, the evaluation of sensitivity and specificity of any diagnostic criteria may be only empirically assessed by using the physician's judgment after an expert panel discussion defines empirical criteria for diagnosis. This assessment was made possible by the community-based Italian Registry of MMM, which has collected incident cases of MMM around Italy since 1999.²³ Among the 475 cases reported to the Registry in the past 4 years, 14 did not fulfill the Italian diagnostic criteria (G Barosi, M Marchetti, unpublished data). For some, alternative diagnosis was established by a panel discussion between pathologists, referring clinicians, and the registry advisory board (e.g., atypi-

Table 7. Updated Cologne clinicopathological criteria for the diagnosis of idiopathic myelofibrosis (IMF).^{21,*}

Clinical Criteria

A1 No preceding or allied subtype of myeloproliferative disorders CML or MDS

A2 Early clinical stages

- Normal hemoglobin or anemia, grade I: hemoglobin \geq 12 g/dL
- Slight or moderate splenomegaly on palpation or $>$ 11 cm on ultrasound scan or CT
- Thrombocytopenia, platelet $>$ $400 \times 10^9/L$

A3 Intermediate clinical stage

- Anemia grade II (hemoglobin \geq 10 g/dL)
- Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes
- Splenomegaly
- No adverse signs[†]

A4 Advanced clinical stage

- Anemia grade III: hemoglobin $<$ 10 g/dL
- 1 or more adverse signs[†]

Pathological Criteria

B1 Megakaryocytic and granulocytic myeloproliferation and relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant-sized megakaryocytes containing clumsy (cloud-like) lobulated nuclei and definitive maturation defects.

Staging of idiopathic myelofibrosis (IMF)

MF0 *prefibrotic stage IMF*: no reticulin fibrosis MF1

MF1 *early IMF*: slight reticulin fibrosis

MF2 *manifest IMF*: marked increase in reticulin and/or collagen fibrosis

MF3 *overt IMF*: advanced collagen fibrosis-osteosclerosis (endophytic bone formation)

*The combination of A1 + B1 establishes IMF—any other criterion confirms IMF.

A1 + A2, B1 + MF0 is consistent with initial (prefibrotic) IMF.

A1 + A3, B1 + MF1, MF2 is consistent with early manifestation of IMF.

A1 + A4, B1 + MF3 is consistent with end stage (full-blown) IMF.

[†] Adverse signs: Age $>$ 70 years, hemoglobin $<$ 10 g/dL, myeloblasts $>$ 2% in peripheral blood, $>$ 2% erythro-normoblasts in PB, leukocytosis $>$ $20 \times 10^9/L$, thrombocytopenia $<$ $300 \times 10^9/L$, severe constitutional symptoms, massive splenomegaly, cytogenetic abnormalities.

Table 8. The Italian criteria for the diagnosis of myelofibrosis with myeloid metaplasia.²²

Necessary Criteria

- A. Diffuse bone marrow fibrosis
- B. Absence of Philadelphia chromosome or BCR-ABL rearrangement in peripheral blood cells

Optional Criteria

1. Splenomegaly of any grade
2. Anisopoikilocytosis with teardrop erythrocytes
3. Presence of circulating immature myeloid cells
4. Presence of circulating erythroblasts
5. Presence of clusters of megakaryoblasts and anomalous megakaryocytes in bone marrow sections
6. Myeloid metaplasia

Diagnosis of MMM is acceptable if the following combinations are present:

- the 2 necessary criteria plus any other 2 optional criteria when splenomegaly is present
- the 2 necessary criteria plus any other 4 optional criteria when splenomegaly is absent

cal chronic myeloid leukemia, mixed myeloproliferative myelodysplastic syndrome, myelodysplastic syndrome with bone marrow fibrosis, spleen lymphoma). In the remaining few cases (2.1% of the whole population of referred cases), the diagnostic criteria were not met, but the panel concluded that they were real cases of MMM (false negative rate). In these few cases, the

limiting constraint for a diagnosis of MMM was the necessary criterion of “presence of diffuse bone marrow fibrosis,” as they presented with none or minimal reticulin fibrosis in the bone marrow or with a fatty bone marrow in which the fibrosis was patchy and localized, representing a previously described pathological variant.²⁴ The rate of false negative cases using the Cologne criteria was 3.5%. These were cases with no clustering of megakaryocytes in the bone marrow or no splenomegaly at diagnosis.

In conclusion, neither a biological hypothesis on the natural history of the disease, nor a nominalistic approach, produces a set of criteria for the diagnosis of MMM that is infallible when compared with the diagnostic skill of expert physicians. This fact, however, does not preclude the enrollment of MMM patients into clinical trials. However, the existence of atypical variants, like those with “prefibrotic myelofibrosis” or “myelofibrosis with fatty bone marrow,” may encompass new diagnostic features that may accommodate histological and clinical heterogeneity.

Prognostic Stratification

Median survival time from diagnosis in the more recent series ranges from 3.5 to 5.5 years, with an actuarial survival rate at 2 and 5 years of 68% and 40%, respectively.²⁵ Among the prognostic systems, the one most used for its simplicity and reliability is the LILLE Scoring System, which defines 3 distinct prognostic

groups on the basis of hemoglobin concentration and WBC at diagnosis.²⁵ In a European collaborative study on 121 patients aged 55 or less,²⁶ the median survival was 128 months, i.e., more than twice that reported in the comprehensive series of MMM patients, documenting that age at presentation is an indicator of prognosis. Anemia, presence of constitutional symptoms, and circulating blasts allowed separation of low-risk and high-risk groups among young patients.

Two biological features have recently challenged the traditional prognostic classifications: the extent of bone marrow neoangiogenesis and the number of circulating progenitor cells. Mesa et al,¹⁶ using the microvessel density values, documented that survival was significantly shorter in patients with a grade 3 or 4 increase in angiogenesis (median survival, 155 months versus 58.6 months).

In 84 cases of MMM, evaluated at different times from the diagnosis, comparing survival in patients who had CD34⁺ levels above and below $300 \times 10^6/L$, death rate was 40% in the former group and 5.1% in the latter, and the difference was significant.⁶ The blast transformation rate was 40% in the group with a high CD34⁺ cell count, while it was 3.4% in the group with a low count, and the difference in time to blast transformation was highly significant. Patients with high CD34⁺ cell count had 50% probability of developing blast transformation at 11 months from the evaluation.

Conventional Therapeutic Options

The conventional therapeutic options for MMM include supportive care, chemotherapy, or biologic-modifying agents. Supportive care entails danazol for the treatment of anemia and/or thrombocytopenia, recombinant erythropoietin for the treatment of anemia and corticosteroids for systemic symptoms. Currently, the standard of chemotherapeutic care in the community for MMM differs among various countries. Hydroxyurea, busulfan, interferon, and low-dose melphalan have been proven to be effective in reducing thrombocytosis and in decreasing the rate of progression of splenomegaly, while minor effects were reported on anemia.

Allogeneic stem cell transplantation (allo-SCT) has usually been used in the setting of advanced and refractory disease, often after the failure of standard therapy. Hematopoietic recovery was reached in 50 out of the 55 patients reported in a collaborative international study.²⁷ Overall, 43.6% of patients died after SCT of infections, chronic graft-versus-host disease (GVHD), acute GVHD, disease progression, solid organ failure, lymphoproliferative disorders, and graft failure. Nineteen patients (34.5%) achieved a complete histological/hematological remission. The Kaplan-Meier esti-

mate of survival at 5 years was 49% with a 57% survival rate for patients receiving an unmanipulated, human leukocyte antigen (HLA)-matched related graft. It is presently unknown to what extent the durable remissions can be attributed to a graft versus leukemia (GVL) effect; however, evidence for a GVL effect comes from donor lymphocyte infusion (DLI)-induced remissions following allo-SCT relapses.

Attempts to reverse a progressive, advanced, rapidly fatal course of the disease (including severe anemia, painful spleen, and thrombocytopenia) have been made with the use of autologous stem cell transplantation. In a Phase II feasibility trial in 17 patients, peripheral blood stem cells (PBSCs) were collected by apheresis at steady state, after granulocyte-colony stimulating factor (G-CSF) alone or after anthracycline/Ara-C chemotherapy and G-CSF in cases of cytogenetic or leukemic progression.²⁸ The median times to neutrophil and platelet recoveries were 21 days (range, 10–34) and 25 days (range, 13 to > 120), respectively. Two patients died of infections at 1 and 18 months post-PBSC transplantation. Reticulin and collagen fibrosis were reduced in 5 of 9 and in 5 of 8 evaluated cases, respectively, at a median of 3 months (range, 1–14) post-PBSC transplantation. Six of 11 red cell transfusion-dependent patients became transfusion-independent (hemoglobin 11–14 g/dL) at a median of 50 days post-PBSC transplantation. Four of 5 patients had a reduction in splenomegaly (by \geq one third palpable span) and 2 of 3 patients had a reduction in bone pain. The maximum duration of response was 39 months.

New Treatments

Reduced-intensity conditioning stem cell transplantation

The use of reduced-intensity conditioning regimen is based on the concept that induction of GVL may be sufficient to obtain disease eradication. In addition, it may reduce transplantation-related mortality and expand the applications of allo-SCT for MMM patients. At the time of this writing (May 2003), 27 patients have been reported in one Phase II, single-institution, prospective study²⁹ and two retrospective cohort studies,^{30,31} comprising a European Group for Blood and Marrow Transplantation (EBMT) survey published in abstract form. Conditioning regimens and source of stem cells were heterogeneous. However, fludarabine was a constant component of the preparative regimen and peripheral blood mobilized stem cells were the most frequent source. Prompt neutrophil and platelet engraftment was reported. In the EBMT survey, the day 21 probability to achieve an absolute neutrophil count (ANC) greater

than $0.5 \times 10^9/L$ was 78%, the day 30 probability to achieve platelet count greater than $20 \times 10^9/L$ was 67%. Acute GVHD was reported in 30% and chronic GVHD in 70% of the patients. Transplantation-related mortality (TRM) was null in the single institution study²⁹ with a follow-up from 4 months to 2.12 years. However, in the EBMT multicenter study, the 1-year TRM was 37% and the relapse rate was 36% at 1 year. In this latter study, 60% had a complete hematologic response with an overall survival of 54% at 1 year. Predictors of survival were acute transformation before SCT and absence of platelet recovery by day +30. Present evidence is not sufficient for comparisons with standard therapy in different risk groups in terms of survival and quality of life. This procedure should be considered a promising therapeutic approach to be used in well-designed clinical trials.

Thalidomide

Thalidomide is thought to exert its therapeutic effect through an antiangiogenic action and modulation of cytokines, particularly tumor necrosis factor- α . In MMM, 6 small Phase II studies published from 2000 to 2002 and a pooled analysis of 5 of these studies documented that thalidomide given at standard dose of 200 to 800 mg a day has a chance of ameliorating anemia, thrombocytopenia, and splenomegaly.³²⁻³⁸ Nevertheless,

most of the patients treated with these doses reported adverse effects that led to a dropout rate of greater than 50% in 3 months. Moreover, untoward increases of white blood cell (WBC) and/or platelet count were frequently reported with clinical consequences like pericardial effusion secondary to myeloid metaplasia.

Recently, Mesa et al³⁹ reported that combining low-dose thalidomide with prednisone produced a better tolerated and equally or more effective than standard dose treatment in 21 patients with MMM. The European Collaboration on MMM has initiated a Phase II trial of low-dose thalidomide as a single agent in 63 patients with MMM using a dose-escalation design and starting with a low dose of thalidomide (M. Marchetti et al, unpublished data). Considering only patients who completed 4 weeks of treatment, 31% had a response: this was mostly due to a beneficial effect of thalidomide on patients with transfusion-dependent anemia, 39% became transfusion independent. Of those MMM patients with moderate to severe thrombocytopenia, 28% increased their platelet count by more than $50 \times 10^9/L$; and 42% of patients with the largest splenomegalies reduced spleen size by more than 2 cm.

These results are not at great variance with those obtained with standard dose thalidomide in MMM patients as pooled from 5 different small trials (**Table 9**).³⁸ By comparing these results with those obtained em-

Table 9. Published studies of thalidomide in myelofibrosis with myeloid metaplasia.

Studies:	Barosi et al (Pooled Analysis of 5 Phase II Studies) ³⁸	Merup et al ³⁷	Mesa et al ³⁹	Marchetti et al (unpublished data, 2003)
n patients	62	15	21	63
Age (median, years)	65	68	63	68
Previous myeloproliferative disease (%)	26	40	28	22
Thalidomide dose, mg/day (minimum/maximum)	100/800	200/800	50/50	50/400
Associated drugs	Various	Various	Prednisone	Various
Transfusion-dependence (patients %)	37	46	48	41
Hemoglobin (median, g/dL)	9.46	10.1	9.0	9.2
Leukocytes (median, $10^9/L$)	7.9	12.6	7.7	7.5
Platelets (median, $10^9/L$)	150	235	154	140
Spleen size (median, cm below costal arc)	13	n.a.	14	13
Dupriez score = 2 (patients %)	25	6	28	19
Drop-out rate at 3 months (patients %)	45	36	5	24
Increase by 2 g/dL of Hb value (patients %)	14	0	45	13
Transfusion interruption (in transfusion-dependent patients, patients %)	30	0	40	39
Increase by $> 50 \times 10^9/L$ of platelet count (patients %)	45	0	76	41
—in patients with a platelet count $< 150 \times 10^9/L$	38	0	55	28
Decrease of spleen size by > 2 cm from costal arc (patients %)	37	0	43	42
Any clinical response (patients %)	n.a.	0	71	66

ploying a fixed low-dose schedule (50 mg per day) of thalidomide combined with prednisone,³⁹ a seemingly higher response rate of anemia and thrombocytopenia was observed with the latter protocol on anemia in nontransfusion-dependent patients. The contribution of prednisone may play a role in this effect since corticosteroids alone, as well as other immunosuppressors, may have an independent effect on anemia and thrombocytopenia in MMM.

Thalidomide is today the most promising drug for patients with symptomatic cytopenias, specially anemia and thrombocytopenia. Its effect on splenomegaly progression has to be established and its efficacy and side effects including the effect on quality of life need to be evaluated in comparative trials against the conventional disease-modifying drugs (e.g., hydroxyurea or interferon).

Imatinib mesylate

Imatinib mesylate (STI571, Gleevec) is a potent and selective tyrosine kinase inhibitor with significant *in vitro* activity against *c-abl* and *bcr-abl*. Imatinib also inhibits 2 other tyrosine kinases: *c-kit* (CD117), which is highly expressed on CD34⁺ cells of patients with MMM; and platelet-derived growth factor-receptor (PDGF-R) that may play a role in the pathogenesis of associated fibrosis.

The evidence on the efficacy of imatinib mesylate in MMM derives from 6 Phase II studies, 4 published in an abstract form, with 87 patients.⁴⁰⁻⁴⁵ Patients were treated with a single daily oral dose of 200 to 400 mg imatinib. The low number of enrolled patients and the not well established enrollment criteria have produced highly variable results. The beneficial effects on splenomegaly ranged from none to marked. In the trial by Cortes et al,⁴⁴ 10 (71%) of 14 patients with splenomegaly had a reduction in spleen size of at least 30% (median reduction, 46%; range, 30%-100%), and 4 patients (29%) with a modest splenomegaly had complete resolution of splenomegaly. Hematologic improvement was noted only in a few patients. In all studies, an undesired increase of WBC or platelet count was reported. These results support the theory that the expression (or overexpression) of *c-kit* or PDGF-R is not by itself sufficient to predict a response to imatinib. The possible role of imatinib in the treatment of early-phase MMM or in combination with other agents with reported activity in this disease, such as thalidomide, deserves further investigation.

Experimental Treatments

Farnesyltransferase inhibitors

R115777 (Zarnestra) is an orally bioavailable farnesyltransferase inhibitor (FTI) that has displayed encouraging activity in patients with acute myeloid leukemia. To determine whether R115777 might exert similar activity in MMM, Mesa et al⁴⁶ evaluated its effects on circulating myeloid progenitor cells from 25 patients using *in vitro* colony-forming assays. The median R115777 concentrations that inhibited colony formation by 50% were, respectively, 34 and 2.7 nM for myeloid and megakaryocytic colonies from MMM patients. Progenitors from normal controls and patients with other myeloproliferative disorders demonstrated similar sensitivity.

The *in vivo* experience of R115777 in MMM derives from one Phase II trial from MD Anderson Cancer Center in myeloproliferative disorders (MPD).⁴⁷ Eight patients with MMM were treated: 2 anemic patients and 4 with splenomegaly responded. Two out of 8 discontinued the drug for toxicity.

SU5416

SU5416 (Z-3-[(2,4-dimethylpyrrol-5-yl) methylidene]-2-indolinone) is a small, lipophilic, highly protein-bound, synthetic receptor tyrosine kinase inhibitor (RTKI) of VEGFR-2, which inhibits the autophosphorylation induced by receptor-ligand binding. SU5416 inhibits VEGF-dependent endothelial cell proliferation *in vitro* and in animal models. In addition to inhibiting VEGFR-2, SU5416 is an RTKI for both *c-kit* and the *fms*-related tyrosine kinase Flk2 (FLT3) receptor. SU5416, therefore, may target bone marrow angiogenesis directly through VEGFR-2 and blast cell proliferation by FLT3 and *c-kit* in patients with MMM. The emerging clinical evidence for the efficacy of SU5416 in MMM is from a multicenter Phase II study, conducted in patients with MPD.⁴⁸ SU5416 was administered at a dose of 145 mg/m² twice a week through a central venous catheter or a peripherally inserted central catheter for a total of 8 infusions in each 4-week cycle. All 3 patients with MMM received 6 or more cycles of therapy. One patient had a partial remission to the first cycle of therapy. One patient, a woman aged 49 with an established history of splenomegaly, anemia, and increasing weakness, had a reduction of more than 50% in splenomegaly and a significant increase in hemoglobin concentration. She also had a major improvement in performance status and was able to return to full-time employment. The other 2 patients with MMM had stable disease throughout the therapy. Frequently observed toxicities consisted primarily of mild-

to-moderate gastrointestinal effects (nausea, diarrhea, emesis, and abdominal pain), headache, fatigue, dyspnea, and catheter site reactions (pain/burning, edema, or tenderness).

REFERENCES

I. Clinical Controversies Involving the Chronic Myeloproliferative Disorders

1. Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood*. 1951;6:372-375.
2. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem-cell and probable clonal origin of the disease. *N Engl J Med*. 1976;295:913-916.
3. Adamson JW, Singer JW, Catalano P, et al. Polycythemia vera. Further in vitro studies of hematopoietic regulation. *J Clin Invest*. 1980;66:1363-1368.
4. Prchal JF, Axelrad AA. Letter: bone-marrow responses in polycythemia vera. *N Engl J Med*. 1974;290:1382.
5. Swolin B, Weinfeld A, Westin J. A prospective long-term cytogenetic study in polycythemia vera in relation to treatment and clinical course. *Blood*. 1988;72:386-395.
6. Diez-Martin JL, Graham DL, Pettit RM, Dewald GW. Chromosome studies in 104 patients with polycythemia vera. *Mayo Clin Proc*. 1991;66:287-299.
7. Berk PD, Goldberg JD, Silverstein MN, et al. Increased incidence of acute leukemia in polycythemia vera associated with chlorambucil therapy. *N Engl J Med*. 1981;304:441-447.
8. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292-2302.
9. Linman J, Bethell F. Agnogenic myeloid metaplasia. *Am J Med*. 1957;22:107-122.
10. Gunz F. Hemorrhagic thrombocytopenia: a critical review. *Blood*. 1960;15:706-722.
11. Taylor KM, Shetta M, Talpaz M, et al. Myeloproliferative disorders: usefulness of X-linked probes in diagnosis. *Leukemia*. 1989;3:419-422.
12. Janssen JW, Anger BR, Drexler HG, Bartram CR, Heimpel H. Essential thrombocytopenia in two sisters originating from different stem cell levels. *Blood*. 1990;75:1633-1636.
13. Roberts BE, Miles DW, Woods CG. Polycythemia vera and myelosclerosis: a bone marrow study. *Br J Haematol*. 1969;16:75-85.
14. Rosenthal N, Bassen F. Course of polycythemia. *Arch Intern Med*. 1938;62:903-917.
15. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocytopenia and a high risk of thrombosis. *N Engl J Med*. 1995;332:1132-1136.
16. Osgood EE. Polycythemia vera: age relationships and survival. *Blood*. 1965;26:243-256.
17. Lawrence JH, Winchell HS, Donald WG. Leukemia in polycythemia vera: relationship to splenic myeloid metaplasia and therapeutic radiation dose. *Ann Intern Med*. 1969;70:763-771.
18. Schwartz SO, Ehrlich L. The relationship of polycythemia vera to leukemia: a critical review. *Acta Med Scand*. 1950;4:129-147.
19. Berlin NI, Louis R. Wasserman and the history of polycythemia vera. *Mt Sinai J Med*. 1995;62:207-215.
20. Osler W. A clinical lecture on erythremia. *Lancet*. 1908;1:143-146.
21. Streiff MB, Smith B, Spivak JL. The diagnosis and manage-

ment of polycythemia vera in the era since the Polycythemia Vera Study Group. *Blood*. 2002;99:1144.

II. Indications for Cytoreductive Therapy in Polycythemia Vera and Essential Thrombocytopenia

1. Berk PD, Goldberg JD, Donovan PB, Fruchtman SM, Berlin NI, Wasserman LR. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. *Semin Hematol*. 1986;23:132-143.
2. Fruchtman SM, Mack K, Kaplan ME, Peterson P, Berk PD, Wasserman LR. From efficacy to safety: a polycythemia vera study group report on hydroxyurea in patients with polycythemia vera. *Semin Hematol*. 1997;34:17-23.
3. Najean Y, Rain JD, for the French Polycythemia Study Group. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. *Blood*. 1997;90:3370-3377.
4. Haanen C, Mathe G, Hayat M. Treatment of polycythemia vera by radiophosphorus or busulphan. A randomized clinical trial. *Br J Cancer*. 1981;44:75-80.
5. Streiff MB, Smith B, Spivak JL. The diagnosis and management of polycythemia vera in the era since the Polycythemia Vera Study Group: a survey of American Society of Hematology members' practice patterns. *Blood*. 2002;99:1144-1149.
6. Gruppo Italiano Studio Polycythemia (GISP). Polycythemia vera: the natural history of 1213 patients followed over 20 years. *Ann Intern Med*. 1995;123:656-664.
7. Silver RT. Interferon-alpha 2b: a new treatment for polycythemia vera. *Ann Intern Med*. 1993;119:1091-1092.
8. Silver RT. Interferon alfa: effects of long-term treatment for polycythemia vera. *Semin Hematol*. 1997;34:40-50.
9. Lengfelder E, Berger U, Hehlmann R. Interferon α in the treatment of polycythemia vera. *Ann Hematol*. 2000;79:103-109.
10. Heis N, Rintelen C, Gisslinger B, et al. The effect of interferon alpha on myeloproliferation and vascular complications in polycythemia vera. *Eur J Haematol*. 1999;62:27-31.
11. Gilbert HS. Long term treatment of myeloproliferative disease with interferon alpha-2b: feasibility and efficacy. *Cancer*. 1998;83:1205-1213.
12. Talpaz M, O'Brien S, Rose E, et al. Phase I study of polyethylene glycol formulation of interferon α -2B (Schering 54031) in Philadelphia chromosome-positive chronic myelogenous leukemia. *Blood*. 2001;98:1708-1713.
13. Tomer A. Effects of anagrelide on in vivo megakaryocyte proliferation and maturation in essential thrombocytopenia. *Blood*. 2002;99:1602-1609.
14. Spivak JL. The optimal management of polycythemia vera. *Br J Haematol*. 2002;116:243-254.
15. Spivak JL. Polycythemia vera: myths, mechanisms, and management. *Blood*. 2002;100:4272-4290.
16. Stobart K, Rogers PC. Allogeneic bone marrow transplantation for an adolescent with polycythemia vera. *Bone Marrow Transplant*. 1994;13:337-339.
17. Jurado M, Deeg H, Gooley T, et al. Haematopoietic stem cell transplantation for advanced polycythemia vera or essential thrombocytopenia. *Br J Haematol*. 2001;112:392-396.
18. Anderson JE, Tefferi A, Craig F, et al. Myeloablation and autologous peripheral blood stem cell rescue results in hematologic and clinical response in patients with myeloid metaplasia with myelofibrosis. *Blood*. 2001;98:586-593.
19. Silver RT. Imatinib mesylate (Gleevec™) reduces phlebotomy requirements in polycythemia vera. *Leukemia*. 2003;17:1186-1187.
20. Barbui T, Barosi G, Grossi A, et al. Evidence- and consensus-based practice guidelines for the therapy of essential

- thrombocytopenia. A statement from the Italian Society of Hematology. *Haematologica*. In press.
21. Tefferi A, Fonseca R, Pereira D, et al. A long-term retrospective study of young women with essential thrombocythemia. *Mayo Clin Proc*. 2001;76:22-28.
 22. Cortelazzo S, Viero P, Finazzi G, et al. Incidence and risk factors for thrombotic complications in a historical cohort of 100 patients with essential thrombocythemia. *J Clin Oncol*. 1990;8:556-562.
 23. Besses C, Cervantes F, Pereira A, et al. Major vascular complications in essential thrombocythemia: a study of the predictive factors in a series of 148 patients. *Leukemia*. 1999;13:150-154.
 24. Harrison CN, Gale RE, Machin SJ, et al. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. *Blood*. 1999;93:417-424.
 25. Teofili L, Pierconti F, Di Febo A, et al. The expression pattern of *c-mpl* in megakaryocytes correlates with thrombotic risk in essential thrombocythemia. *Blood*. 2002;100:714-717.
 26. Ruggeri M, Gisslinger H, Tosetto A, et al. Factor V Leiden mutation carriership and venous thromboembolism in polycythemia vera and essential thrombocythemia. *Am J Hematol*. 2002;71:1-6.
 27. Harrison CN, Donohue S, Carr P, et al. Patients with essential thrombocythemia have an increased prevalence of antiphospholipid antibodies which may be associated with thrombosis. *Thromb Haemost*. 2002;87:802-807.
 28. Jantunen R, Juvonen E, Ikkala E. The predictive value of vascular risk factors and gender for the development of thrombotic complications in essential thrombocythemia. *Ann Hematol*. 2001;80:74-78.
 29. van Genderen PJ, Michiels JJ. Erythromelalgic, thrombotic and hemorrhagic manifestations of thrombocythemia. *Presse Med*. 1994;23:73-77.
 30. van Genderen PJ, Budde U, Michiels JJ, et al. The reduction of large vonWillebrand multimers in plasma in essential thrombocythemia is related to the platelet count. *Br J Haematol*. 1996;93:962-965.
 31. Cervantes F, Alvarez-Larran A, Talarin C, et al. Myelofibrosis with myeloid metaplasia following essential thrombocythemia: actuarial probability, presenting characteristics and evolution in a series of 195 patients. *Br J Haematol*. 2002;118:786-790.
 32. Andersson PO, Ridell B, Wadenvik H, Kutti J. Leukemic transformation of essential thrombocythemia without previous cytoreductive treatment. *Ann Hematol*. 2000;79:40-42.
 33. Tefferi A. Risk-based management in essential thrombocythemia. In: Broudy V, ed. *Hematology 1999*. American Society of Hematology Education Program Book. Washington, DC: American Society of Hematology. 1999;172-177.
 34. Gugliotta L, Marchioli R, Fiacchini M, et al. Epidemiological, diagnostic, therapeutic and prognostic aspects of essential thrombocythemia in a retrospective study of the GIMMC group in two thousand patients [abstract]. *Blood*. 1997;90(suppl 1):348a.
 35. Barbui T, Finazzi G. Treatment indications and choice of a platelet-lowering agent in essential thrombocythemia. *Curr Hematol Rep*. 2003;2:248-256.
 36. Ruggeri M, Finazzi G, Tosetto A, et al. No treatment for low-risk essential thrombocythemia: results from a prospective study. *Br J Haematol*. 1998;103:772-777.
 37. Randi ML, Luzzatto G, Fabris F. Low-risk thrombocythemia in patients younger than 40. *Br J Haematol*. 1999;104:928-936.
 38. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea in the treatment of patients with essential thrombocythemia at high risk of thrombosis: a prospective randomized trial. *N Engl J Med*. 1995;332:1132-1136.
 39. Finazzi G, Ruggeri M, Rodeghiero F, et al. Second malignancies in patients with essential thrombocythemia treated with busulphan and hydroxyurea: long-term follow-up of a randomized clinical trial. *Br J Haematol*. 2000;110:577-583.
 40. Sterkers Y, Preudhomme C, Lai J-L et al. Acute myeloid leukemia and myelodysplastic syndromes following essential thrombocythemia treated with hydroxyurea: high proportion of cases with 17p deletion. *Blood*. 1998;91:616-622.
 41. Finazzi G, Ruggeri M, Rodeghiero F, Barbui T. Efficacy and safety of long-term use of hydroxyurea in young patients with essential thrombocythemia and a high risk of thrombosis [letter]. *Blood*. 2003;101:3749.
 42. Lofvenberg E, Nordenson I, Wahlin A. Cytogenetic abnormalities and leukemic transformation in hydroxyurea-treated patients with Philadelphia chromosome negative chronic myeloproliferative disease. *Cancer Genet Cytogenet*. 1990;49:57-67.
 43. Murphy S, Iland H, Rosenthal D et al. Essential thrombocythemia: an interim report from the Polycythemia Vera Study Group. *Semin Hematol*. 1986;23:177-182.
 44. Hanft VN, Fruchtman SR, Pickens CV, et al. Acquired DNA mutations associated with in vivo hydroxyurea exposure. *Blood*. 2000;95:3589-3593.
 45. Anagrelide Study Group. Anagrelide, a therapy for thrombocytopenic states: experience in 577 patients. *Am J Med*. 1992;92:69-76.
 46. Fruchtman SM, Pettitt RM, Gilbert HS, et al. Anagrelide: analysis of long term safety and leukemogenic potential in myeloproliferative diseases. *Blood*. 2002;100:70a.
 47. Storen EC, Tefferi A. Long-term use of anagrelide in young patients with essential thrombocythemia. *Blood*. 2001;97:863-866.
 48. Lengfelder E, Griesshammer M, Hehlmann R. Interferon-alpha in the treatment of essential thrombocythemia. *Leuk Lymphoma*. 1996;22(suppl 1):135-142.
 49. Griesshammer M, Bergmann L, Pearson TC. Fertility, pregnancy and the management of myeloproliferative disorders. In: Green AR, Pearson TC, eds. *Myeloproliferative Disorders*. London: Bailliere's Clinical Haematology; 1998:859-874.
 50. Wright CA, Tefferi A. A single institutional experience with 43 pregnancies in essential thrombocythemia. *Eur J Haematol*. 2001;66:152-159.

III. The Use of Aspirin in Polycythemia Vera

1. Brusamolino E, Salvaneschi L, Canevari A, Bernasconi C. Efficacy trial of pipobroman in polycythemia vera and incidence of acute leukemia. *J Clin Oncol*. 1984;2:558-561.
2. Najean Y, Deschamps A, Dresch C, Daniel MT, Rain JD, Arrago JP. Acute leukemia and myelodysplasia in polycythemia vera. A clinical study with long-term follow-up. *Cancer*. 1988;61:89-95.
3. Najean Y, Dresch C, Rain JD. Intermediary analysis of a French protocol of treatment of polycythemias (1980-1990): 253 patients. The French Group for the Study of Polycythemias. *Nouv Rev Fr Hematol*. 1991;33:201-203.
4. Lofvenberg E, Wahlin A. Management of polycythemia vera, essential thrombocythemia and myelofibrosis with hydroxyurea. *Lofvenberg E Eur J Hematol*. 1988;41:375-381.
5. Weinfeld A, Swolin B, Westin J. Acute leukaemia after hydroxyurea therapy in polycythemia vera and allied disorders: prospective study of efficacy and leukaemogenicity

- with therapeutic implications. *Eur J Hematol.* 1994;52:134-139.
6. Lofvenberg E, Nordenson I, Wahlin A. Cytogenetic abnormalities and leukemic transformation in hydroxyurea-treated patients with Philadelphia chromosome negative chronic myeloproliferative disease. Lofvenberg E *Cancer Genet Cytogenet.* 1990;49:57-67.
 7. Najean Y. Treatment of polycythemia vera with hydroxyurea or pipobroman. Efficacy and toxicity analysed from a protocol of 96 patients under 65 years of age. *Najean Y Press Med.* 1992;21:1753-1757.
 8. Messinezy M, Pearson TC, Prochazka A, Wetherley-Mein G. Treatment of primary proliferative polycythaemia by venesection and low dose busulphan: retrospective study from one centre. *Br J Haematol.* 1985;61:657-666.
 9. Rozman C, Giralt M, Feliu E, Rubio D, Cortes MT. Life expectancy of patients with chronic nonleukemic myeloproliferative disorders. *Cancer.* 1991;67:2658-2663.
 10. Brandt L, Anderson H. Survival and risk of leukaemia in polycythaemia vera and essential thrombocythaemia treated with oral radiophosphorus: are safer drugs available? *Eur J Haematol.* 1995;54:21-26.
 11. Gruppo Italiano Studio Policitemia (GISP). Polycythemia vera: the natural history of 1213 patients followed over 20 years. *Ann Intern Med.* 1995;123:656-664.
 12. Berk PD, Goldberg JD, Donovan PB, et al. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. *Semin Hematol.* 1986;23:132-143.
 13. Haanen C, Mathe G, Hayat M. Treatment of polycythaemia vera by radiophosphorus or busulphan: A randomized clinical trial. *Br J Cancer.* 1981;44:75.
 14. Najean Y, Rain JD for the French Polycythemia Study Group. Treatment of polycythemia vera: use of ³²P alone or in combination with maintenance therapy using hydroxyurea in 461 patients greater than 65 years of age. *Blood.* 1989;69:2319.
 15. Najean Y, Rain JD for the French Polycythemia Study Group. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. *Blood.* 1997;90:3370.
 16. Tartaglia AP, Goldberg JD, Berk PD, Wasserman LR. Adverse effects of antiaggregating platelet therapy in the treatment of polycythemia vera. *Semin Hematol.* 1986;23:172.
 17. Gruppo Italiano Studio Policitemia. Low-dose aspirin in polycythemia vera: A pilot study. *Br J Haematol.* 1997;97:453.
 18. Landolfi R, Ciabattini G, Patrignani P, et al. Increased Thromboxane biosynthesis in patients with polycythemia vera: evidence for aspirin-suppressible platelet activation in vivo. *Blood.* 1992;80:1965-1971.
 19. Tefferi A. Pathogenic mechanisms in chronic myeloproliferative disorders: polycythemia vera, essential thrombocytopenia, agnogenic myeloid metaplasia, and chronic myelogenous leukemia. *Semin Haematol.* 1999;36:3-8.
 20. Landolfi R, Marchioli R, Patrono C. Mechanisms of bleeding and thrombosis in myeloproliferative disorders. *Thromb Haemost.* 1997;77:622-626.
 21. Chievitz E, Thiede T. Complications and causes of death in polycythemia vera. *Acta Medica Scandinavica.* 1962;172:513-523.
 22. Landolfi R, Marchioli R, on behalf of the ECLAP Investigators. European Collaboration on Low-dose Aspirin in Polycythemia vera (ECLAP): a randomized trial. *Semin Thromb Haemost.* 1997;23:473-478.
 23. Marchioli R, Barbui T, Landolfi R, Tognoni G. Feasibility of randomised clinical trials in rare diseases: the case of polycythemia vera. *Leuk Lymphoma.* 1996;22:S121-S127.
 24. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *Br Med J.* 2002;324:71-86.

IV. Myelofibrosis with Myeloid Metaplasia: An Update

1. Reeder TL, Bailey RJ, Dewald GW, Tefferi A. Both B and T lymphocytes may be clonally involved in myelofibrosis with myeloid metaplasia. *Blood.* 2003;101:1981-1983.
2. Le Bousse-Kerdiles MC, Martyré MC, and the French INSERM Research Network on Myelofibrosis with Myeloid Metaplasia. Myelofibrosis: pathogenesis of myelofibrosis with myeloid metaplasia. *Springer Semin Immunopathol.* 2000;21:491-509.
3. Thiele J, Kvasnicka HM, Czeslick C. CD34⁺ progenitor cells in idiopathic (primary) myelofibrosis: a comparative quantification between spleen and bone marrow tissue. *Ann Hematol.* 2002;81:86-89.
4. Brouty-Boyé D, Doucet C, Clay D, Le Bousse-Kerdiles MC, Lampidis TJ, Azzarone B. Phenotypic diversity in human fibroblasts from myelometaplastic and non myelometaplastic hemopoietic tissues. *Int J Cancer.* 1998;76:767-773.
5. Briard D, Brouty-Boyé D, Giron-Michel J, Azzarone B, Jasmin C, Le Bousse-Kerdiles MC. Impaired NK cell differentiation of blood-derived CD34⁺ progenitors from patients with myeloid metaplasia with myelofibrosis. *Clin Immunol.* 2003;106:201-212.
6. Barosi G, Viarengo G, Pecci A et al. Diagnostic and clinical relevance of the number of circulating CD34⁺ cells in myelofibrosis with myeloid metaplasia. *Blood.* 2001;98:3249-3255.
7. Moliterno AR, Spivak JL. Posttranslational processing of the thrombopoietin receptor is impaired in polycythemia vera. *Blood.* 1999;94:2555-2561.
8. Elliot MA, Yoon SY, Kao P, Li CY, Tefferi A. Simultaneous measurement of serum thrombopoietin and expression of megakaryocyte c-Mpl with clinical and laboratory correlates for myelofibrosis with myeloid metaplasia. *Eur J Haematol.* 2002;68:175-179.
9. Frey BM, Rafii S, Teterson M, Eaton D, Crystal RG, Moore MA. Adenovector-mediated expression of human thrombopoietin cDNA in immune-compromised mice: insight into the pathophysiology of osteomyelofibrosis. *J Immunol.* 1998;160:691-699.
10. Rameshwar P, Narayanan R, Qian J, Denny TN, Colon C, Gascon P. NF-kappa B as a central mediator in the induction of TGF-beta in monocytes from patients with idiopathic myelofibrosis: an inflammatory response beyond the realm of homeostasis. *J Immunol.* 2000;165:2271-2277.
11. Chagraoui H, Komura E, Tulliez M, Vainchenker W, Wendling F. Prominent role of TGF-β1 secreted by hematopoietic cells in bone marrow fibrosis induction. *Blood.* 2002;100:3495-3503.
12. Schmitt A, Drouin A, Masse JM, Guichard J, Shagraoui H, Cramer EM. Polymorphonuclear neutrophil and megakaryocyte mutual involvement in myelofibrosis pathogenesis. *Leuk Lymphoma.* 2002;43:719-724.
13. Chagraoui H, Tulliez M, Smayra T, et al. Stimulation of osteoprotegerin production is responsible for osteosclerosis in mice overexpressing TPO. *Blood.* 2003;101:2983-2989.
14. Vannucchi AM, Bianchi L, Cellai C, et al. Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1(low) mice). *Blood.* 2002;100:1123-1132.
15. Migliaccio AR, Rana RA, Sanchez M, et al. GATA-1 as a regulator of mast cell differentiation revealed by the pheno-

- type of the GATA-1 low mouse mutant. *J Exp Med*. 2003;197(3):281-296.
16. Mesa RA, Hanson CA, Rajkumar SV, Schroeder G, Tefferi A. Evaluation and clinical correlations of bone marrow angiogenesis in myelofibrosis with myeloid metaplasia. *Blood*. 2000;96:3374-3380.
 17. Di Raimondo F, Azzaro MP, Palumbo GA, et al. Elevated vascular endothelial growth factor (VEGF) serum levels in idiopathic myelofibrosis. *Leukemia*. 2001;15:976-980.
 18. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34 (+) cells identifies a population of functional endothelial precursors. *Blood*. 2000;95:952-958.
 19. Laszlo J. Myeloproliferative disorders (MPD): myelofibrosis, myelosclerosis, extramedullary hematopoiesis, undifferentiated MPD, and hemorrhagic thrombocythemia. *Semin Hematol*. 1975;12:409-432.
 20. Thiele J, Kvasnicka HM, Diehl V, Fischer R, Michiels J. Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disorders by histopathology, histochemistry and immunostaining from the bone marrow. *Leuk Lymphoma*. 1999;33:207-218.
 21. Michiels JJ, Thiele J. Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera, and idiopathic myelofibrosis (agnogenic myeloid metaplasia). *Int J Hematol*. 2002;76:133-145.
 22. Barosi G, Ambrosetti A, Finelli C, et al. The Italian Consensus Conference on diagnostic criteria for myelofibrosis with myeloid metaplasia. *Br J Haematol*. 1999;104:730-737.
 23. Barosi G, Marchetti M, Azzan C, for the Italian Registry of Myelofibrosis with Myeloid Metaplasia. The Italian Registry of Myelofibrosis is one year old. *Haematologica*. 2000;85(11):1121-1122.
 24. Polino G, Barosi G, Berzuini A, et al. Fatty bone marrow with severe myeloid hypoplasia in idiopathic myelofibrosis. *Haematologica*. 1986;71:117-121.
 25. Dupriez B, Morel P, Demory JL, et al. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. *Blood*. 1996;88:1013-1018.
 26. Cervantes F, Barosi G, Demory JL, et al. Myelofibrosis with myeloid metaplasia in young individuals: disease characteristics, prognostic factors and identification of risk groups. *Br J Haematol*. 1998;102:684-690.
 27. Guardiola P, Anderson JE, Bandini G, et al. Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Societe Francaise de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study. *Blood*. 1999;93:2831-2838.
 28. Anderson JE, Tefferi A, Craig F, et al. Myeloablation and autologous peripheral blood stem cell rescue results in hematologic and clinical responses in patients with myeloid metaplasia with myelofibrosis. *Blood*. 2001;98:586-593.
 29. Devine SM, Hoffman R, Verma A, et al. Allogeneic blood cell transplantation following reduced-intensity conditioning is effective therapy for older patients with myelofibrosis with myeloid metaplasia. *Blood*. 2002;99:2255-2258.
 30. Hessling J, Kroger N, Werner M, et al. Dose-reduced conditioning regimen followed by allogeneic stem cell transplantation in patients with myelofibrosis with myeloid metaplasia. *Br J Haematol*. 2002;119:769-772.
 31. Hertenstein B, Guardiola P, Finke J, et al. Non-myeloablative (NMA) stem cell transplantation (SCT) for myeloid metaplasia with myelofibrosis (MMM). A survey from the chronic leukemia Working Party of the EBMT [abstract]. *Blood*. 2002;100:70a.
 32. Barosi G, Grossi A, Comotti B, Musto P, Gamba G, Marchetti M. Safety and efficacy of thalidomide in patients with myelofibrosis with myeloid metaplasia. *Br J Haematol*. 2001;114:78-83.
 33. Canepa L, Ballerini F, Valardo R, et al. Thalidomide in agnogenic and secondary myelofibrosis. *Br J Haematol*. 2001;115:313-315.
 34. Elliott MA, Mesa RA, Li CY, et al. Thalidomide treatment in myelofibrosis with myeloid metaplasia. *Br J Haematol*. 2002;117:288-296.
 35. Piccaluga PP, Visani G, Pileri SA, et al. Clinical efficacy and antiangiogenic activity of thalidomide in myelofibrosis with myeloid metaplasia: a pilot study. *Leukemia*. 2002;16:1609-1614.
 36. Pozzato G, Zorat F, Nascimben F, Comar C, Kikic F, Festini G. Thalidomide therapy in compensated and decompensated myelofibrosis with myeloid metaplasia. *Haematologica*. 2001;86:772-773.
 37. Merup M, Kutti J, Birgergard G, et al. Negligible clinical effects of thalidomide in patients with myelofibrosis with myeloid metaplasia. *Med Oncol*. 2002;19:79-86.
 38. Barosi G, Elliott M, Canepa L, et al. Thalidomide in myelofibrosis with myeloid metaplasia: a pooled-analysis of individual patient data from 5 studies. *Leuk Lymph*. 2002;43:2301-2307.
 39. Mesa RA, Steensma DP, Pardanani A, et al. A Phase II trial of combination low-dose thalidomide and prednisone for the treatment of myelofibrosis with myeloid metaplasia. *Blood*. 2003;101:2534-2541.
 40. Tefferi A, Mesa RA, Gray LA, et al. Phase 2 trial of imatinib mesylate in myelofibrosis with myeloid metaplasia. *Blood*. 2002;99:3854-3856.
 41. Ho AYL, Lim S, Fishlock K, et al. Imatinib mesylate in myelofibrosis: preliminary results show early sustained improvements in platelet counts and splenomegaly [abstract]. *Blood*. 2002;100:799a.
 42. Gisslinger H, Gisslinger B, Kees M, et al. Imatinib mesylate in chronic idiopathic myelofibrosis, a Phase II trial [abstract]. *Blood*. 2002;100:800a.
 43. Hasselblach HC, Bjerrum OW, Jensen BA, Hansen PB, Birgens H. ST1571 (Gleevec) therapy in idiopathic and postpolycythemic myelofibrosis. *Blood*. 2002;100:344b.
 44. Cortes J, Giles F, O'Brien S, et al. Results of imatinib mesylate therapy in patients with refractory or recurrent acute myeloid leukemia, high-risk myelodysplastic syndrome, and myeloproliferative disorders. *Cancer*. 2003;97:2760-2766.
 45. Odenike O, Hoving K, Sher H, et al. Phase II study of imatinib mesylate (IM) in myelofibrosis with myeloid metaplasia (MMM). In: Abstracts of 2003 ASCO Annual Meeting; May 31-June 3, 2003; Chicago, Illinois. Abstract 2354.
 46. Mesa RA, Tefferi A, Gray LA, Reeder T, Schroeder G, Kaufmann SH. In vitro antiproliferative activity of the farnesyltransferase inhibitor R115777 in hematopoietic progenitors from patients with myelofibrosis with myeloid metaplasia. *Leukemia*. 2003;17:849-855.
 47. Cortes J, Albitar M, Thomas D, et al. Efficacy of the farnesyl transferase inhibitor R115777 on chronic myeloid leukemia and other hematologic malignancies. *Blood*. 2003;101:1692-1697.
 48. Giles FJ, Cooper MA, Silverman L, et al. Phase II study of SU5416—a small molecule, vascular endothelial growth factor tyrosine-kinase receptor inhibitor in patients with refractory myeloproliferative diseases. *Cancer*. 2003;97:1920-1928.