

June 2004

Faculty

Steven Fruchtman, MD

Mount Sinai School of
Medicine
New York, NY

Giovanni Barosi, MD

IRCCS Policlinico S. Matteo
Pavia, Italy

John J. Fung, MD

University of Pittsburgh
Pittsburgh, PA

Philippe Guardiola, MD

Fred Hutchinson Cancer
Research Center
Seattle, WA

Josef Prchal, MD

Baylor College of Medicine
Houston, TX

Jacob Rand, MD

Albert Einstein College of
Medicine
Bronx, NY

Jerry L. Spivak, MD

Johns Hopkins University
School of Medicine
Baltimore, MD

CLINICAL SYMPOSIA REPORT

Myeloproliferative Disorders: Issues, Controversies, and Advances in Treatment

Abstract

Myeloproliferative disorders (MPD) are typically, but not invariably, clonal disorders arising in a multipotent hematopoietic stem cell. For reasons not understood, in these disorders there is a proclivity toward increased cell proliferation and marrow collagen deposition and, although influenced by therapy, a tendency to transformation into acute leukemia. The major causes of death in Philadelphia-negative (Ph-) MPD (polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis) are thrombotic or hemorrhagic complications and leukemic transformation. To be successful, any therapeutic modalities investigated must influence these outcomes that lead to death. The primary treatment goals for Ph- MPD are to ameliorate symptoms, reduce thrombotic complications, and suppress, modify, or eliminate the abnormal clone. In such efforts, however, the side effects from the therapies must be minimized and, in particular, leukemic transformation must be avoided. In addition to medical therapy, the Budd-Chiari syndrome in MPD patients has an option of surgical intervention. Issues of treatment, controversies, and future directions for the management of Ph- MPD are addressed herein.

Based on symposia held in December 2003, San Diego, CA

Myeloproliferative Disorders: Issues, Controversies, and Advances in Treatment

Introduction

The historic Polycythemia Vera Study Group (PVSG) 01 trial emphasized the fact that the therapies used in the management of Philadelphia-negative myeloproliferative disorders (Ph-MPD) may potentiate leukemic conversion.¹ This trial showed that polycythemia vera (PV) patients who were treated with phlebotomy alone had the best long-term outcomes. This finding was explained by the observation that until year 7, the incidences of acute leukemia in the 3 arms (chlorambucil, ³²P, and phlebotomy) of that historic trial were similar. However, after 7 years the incidence of acute leukemia started to increase among patients who were treated with chlorambucil or with ³²P. As a result, chlorambucil is no longer used for PV. In this particular trial, 34% of patients died of leukemia; the other major cause of death was thrombosis.¹ The goals in treating MPD patients include reduction of symptoms and of both thrombotic and hemorrhagic events, and, ideally, restoration of normal polyclonal hematopoiesis. These goals are not yet achievable in Ph-MPD.

Diagnosis of Myeloproliferative Disorders

The controversy over the diagnosis of MPD, especially PV, ET and their treatment, stems from the fact that the molecular basis of these disorders is unknown. Diagnostic and mechanistic difficulties associated with the chronic MPD include variable clinical manifestations and phenotypic mimicry between them and benign or nonclonal hematopoietic disorders. With the exception of chronic myeloid leukemia (CML), there is no specific clinically applicable clonal marker available. Furthermore, their cause is unknown. Over 30 years ago, the PVSG created guidelines for the diagnosis of patients for clinical trials.² However, protocols for clinical trials do not always reflect daily clinical practice. To determine what tests are actually used to diagnose PV and what treatments are prescribed, hematologists from the American Society of Hematology (ASH) were polled as to their practices with respect to the diagnosis and management of PV patients. It was apparent from this survey that there was no consensus as to the best way to diagnose or to treat PV.³

The major diagnostic issue in testing for PV is distinguishing it from other forms of erythrocytosis, which probably occur at a greater frequency than PV. Several tests have been used (Table 1): (1) assay of serum erythropoietin (EPO), which is not sensitive; (2) cytogenetics, which are not particularly useful because less than 30% of patients have abnormal cytogenetics; (3) clonality assays, which are only informative in women; (4) bone marrow morphology and histology, which in PV patients can be normal and are generally nonspecific; (5) erythroid progenitor cell as-

Table 1. Diagnostic Tests for Polycythemia Vera

Measurement	Outcome
Serum erythropoietin	Not sensitive; low negative predictive value
Cytogenetics	Abnormal in less than 25% of patients at diagnosis
Clonal assays	Applicable only in informative women; not sensitive
Bone marrow morphology	Can be normal; not specific
Erythroid progenitor cell assays	Not widely available, not standardized, not sensitive
Computed tomography scanning for spleen size	Not standardized, not specific
Red blood cell mass	Raised red cell mass in conjunction with other criteria is confirmative of polycythemia vera

says, which are not widely available, not standardized, and not sensitive; and (6) computed tomography (CT) scanning or ultrasound for splenomegaly, which has not been standardized. EPO levels measured in PV patients are often below the lower limit of normal as compared to patients with known secondary erythrocytosis and pseudoerythrocytosis. However, there are secondary erythrocytosis and PV patients with EPO levels in the normal range, in which case the assay does not distinguish PV from secondary erythrocytosis.

The formation of endogenous erythroid colonies *in vitro* is a feature of PV. But this can also occur not only in the other MPD, but also at a low level in normal individuals, and it can be absent in PV patients.⁴ In a study of endogenous erythroid colony formation in PV progenitor cells that had been previously exposed to EPO and then placed in culture medium without EPO, only 37% of the cells formed colonies in the absence of EPO.⁵ If the same cells were never exposed to EPO, initially only 21% survived to form EPO-independent colonies. Thus, from the total number of PV cells, 63% with previous EPO exposure and 79% without any EPO exposure did not form endogenous erythroid colonies. Another study in PV patients confirmed the finding that when endogenous erythroid colony formation was measured, only 34% of the PV progenitor cells formed erythroid colonies in the absence of EPO, but 60% of the cells had trisomy 8.⁶ Thus, the vast majority of the erythroid precursor cells in PV are EPO-dependent. A paper by Oehlers et al reported that imatinib mesylate affected EPO-independent cells from PV patients but not the EPO-dependent cells,⁷ but as indicated above, the former are only a small percentage of the erythroid precursor cell pool.⁸ From these data it is apparent that there is heterogeneity in disease expression or variable pen-

etrance with respect to endogenous erythroid colony formation. Since erythrocytosis is the only feature that separates PV from its companion MPD, and since the hematocrit is not an accurate indicator of the red blood cell mass, measurement of the red cell mass should not be abandoned for surrogate markers that cannot address critical diagnostic or clinical management issues.

With respect to clonality, while all patients with CML are clonal, PV, ET, and idiopathic myelofibrosis (IMF) patients may be either clonal or nonclonal.⁹ This could be due to disease heterogeneity, lack of sensitivity in the assays used, or skewing due to age. Finally, cytogenetic markers in the bone marrow may be absent in circulating peripheral blood granulocytes.¹⁰⁻¹² Thus, the clinical tests that are currently employed as diagnostic tools in PV are not foolproof and new biomarkers such as impaired platelet Mpl expression or overexpression of granulocyte PRV-1 mRNA may provide additional diagnostic assistance.

Thrombopoietin Receptor Expression in the MPD

Platelets from patients with PV, ET, and IMF frequently have impaired expression of the thrombopoietin (TPO) receptor, Mpl. PV and ET megakaryocytes also have low levels of Mpl in contrast to those in patients with secondary erythrocytosis.^{13,14} Additionally, CD34 positive cells from patients with PV also have impaired Mpl expression. They also show defects in TPO-mediated signal transduction compared to immunophenotypically similar normal CD34 positive cells and do not proliferate well in the presence of TPO. The Mpl defect follows the disease; as the disease becomes more severe with time, Mpl expression decreases further. Platelet Mpl expression could be used as a test to distinguish PV from secondary forms of erythrocytosis. However, since this defect also occurs in ET,^{15,16} the defect cannot be used to distinguish among PV, ET, and IMF.¹⁷ Using quantitative densitometry in 83 PV, 12 IMF, and 75 ET patients, 78% of PV, 75% of IMF, and 45% of ET patients had impaired c-Mpl expression, and there was gender heterogeneity in each of these diseases. A low platelet Mpl level in ET patients might, interestingly, have prognostic significance since ET patients with reduced megakaryocyte Mpl had an increased propensity for thrombosis compared to patients with secondary thrombocytosis.¹⁸ In addition, some patients diagnosed with ET who had low platelet Mpl expression later developed either PV or IMF.¹³ Thus, this biomarker may have clinical significance even though the biologic basis for these associations has not been determined.

Recently, on sequencing the Mpl receptor gene, a single amino acid substitution, lysine for an asparagine at position 39 in exon 2, was found that was associated with impaired Mpl expression. From the patient pool at Johns Hopkins, it was found that 7–8% of the African American population had this polymorphism. Some of the heterozygotes for this receptor defect had thrombocytosis, as did 2 homozygotes, but penetrance was not always present. (Moliterno et al, *PNAS* [in press] 2004).

Abnormal Granulocyte Expression of PRV-1 mRNA in the MPD

Abnormal PRV-1 expression was discovered by subtractive hybridization using PV and normal granulocyte mRNA. The

gene encoding for PRV-1 was described first in 1977 and its protein was given the cluster designation CD177. It belongs to the uPAR receptor superfamily.¹⁹ PRV-1 is one allele of CD177 and the other, differing by one amino acid, is NB1, which causes transfusion-related acute lung injury and neonatal and drug-induced immune neutropenia. Interestingly, neutrophil expression of CD177 is genetically controlled and gender-related. Women make more of this protein than men. The gene for CD177 is structurally normal in PV.²⁰ PRV-1 transcription is increased in circulating neutrophils in PV patients and in some patients with IMF and ET but not in CML or secondary erythrocytosis. PRV-1 transcription is not differentially increased in the bone marrow hematopoietic progenitor cells of MPD patients. A recent study, using real time PCR on bone marrow cells, reported no difference in the expression of PRV-1 in patients who had reactive states, like anemia or thrombocytopenia, and myelodysplastic syndromes, ET, CML, IMF, and PV.²¹ Granulocyte colony stimulating factor (G-CSF) and hydroxyurea (HU) upregulated PRV-1 expression, whereas interferon downregulated it.²² When PRV-1 levels were measured in peripheral blood granulocytes, PV patients had significantly higher levels of PRV-1 in comparison to healthy patients or patients with secondary erythrocytosis.²³ Other data, however, show overlap between PV patients and patients with congenital PV, ET, and normal controls.²⁴ While there does appear to be concordance between elevated PRV-1, clonality, and endogenous colony formation in PV, 4 classical PV patients have been described with normal PRV-1 levels.²⁴ At the present time, if granulocytic PRV-1 overexpression is present, then an MPD is highly likely. However, if PRV-1 is not overexpressed, then an MPD cannot be excluded. Interestingly, abnormal expression of the PRV-1 gene in ET also appears to increase the propensity for thrombosis, although not concordantly with impaired Mpl expression²⁵ and for the transition between ET and PV.²⁶ There appears to be much heterogeneity with respect to PRV-1 overexpression in the chronic MPD. Depending on the laboratory performing the assay, the range of positivity was 69–100 % of patients with PV, 50–100 % for IMF patients, and 33–67% for ET patients. Similarly, impaired platelet Mpl expression was observed in 30–95% of PV patients, 67–75% of IMF patients, and 35–68% of ET patients; as mentioned above, there was also no concordance between the 2 abnormalities in ET patients.

The basis for this biomarker heterogeneity is unknown. A Spanish family with different members in successive generations having ET, PV or IMF may shed some light on the issue.²⁷ This family pedigree suggests that these disorders involve a common hematopoietic stem cell, but that expression of the genetic defect varies in a given patient. In another study that evaluated progenitor cell behavior in ET patients, the investigators measured EPO independence, cell cycle activity, and clonality. Only 14 of the 22 ET patients were concordant for these abnormalities while 8 were not, indicating heterogeneity in expression of specific biologic abnormalities in the progenitor cells of a single MPD.²⁸

With respect to diagnosis, erythrocytosis is the only diagnostic feature unique to PV amongst the MPD. For IMF, elevation

in circulating CD34 positive cells is diagnostic, while isolated thrombocytosis is the only unique feature of ET. From the above discussion of heterogeneity of disease biology, a single molecular marker cannot be expected to substitute for clinical observations; measurement of the red cell mass and plasma volume as the following case demonstrates is mandatory if PV is a diagnostic consideration. For example, a patient was seen with massive splenomegaly and elevated platelet and white cell counts with marrow fibrosis but with a normal hemoglobin level. A normal hemoglobin level would be unusual with massive splenomegaly and on further evaluation she was found to have a red cell mass of 52 mL/kg (normal: 20–30 mL/kg) and a plasma volume of 71 mL/kg (normal: 30–45 mL/kg). Thus, she had an expanded plasma volume as a consequence of the splenomegaly masking elevation of the red cell mass. Her diagnosis was actually PV and not IMF. It is also clear in this patient that an abnormal biomarker study, either elevated PRV-1 or Mpl, would not have discriminated between PV, IMF, or ET. Only a red cell mass study and, if normal, a bone marrow biopsy could make the essential diagnostic distinctions.

Polycythemias

Polycythemia, a condition in which there are too many red blood cells, can be classified as either primary or secondary. Primary polycythemic disorders are those in which mutations are either acquired or congenital²⁹ and are expressed within stem cells or progenitor cells, which drive the eventual accumulation of red cells. Whether this occurs from increased proliferation or decreased apoptosis is not yet known.

In contrast, the secondary polycythemic disorders may also be acquired or congenital. However, they are driven by circulating factors independent of the function of hematopoietic stem cells. These factors include EPO, insulin-like growth factor (IGF-1), or cobalt.^{30,31} Some patients who have had bilateral nephrectomy, a major site of EPO production, have very low EPO levels, but high levels of IGF-1, a well-known stimulator of erythropoiesis.³² Similarly, there are well-defined examples of polycythemic states that result from cobalt toxicity. Thus, both primary and secondary polycythemias could be either acquired or congenital.

Acquired polycythemias result from high altitude, lung disease, and PV. Congenital polycythemias may be either primary or secondary. The first examples of congenital polycythemias, which are very rare, were individuals with high affinity hemoglobins. An even rarer disorder, a diphosphoglyceromutase deficiency, causes decreased levels of 2,3-diphosphoglycerate.³³ This metabolic intermediate facilitates removal of oxygen from hemoglobin.

The low EPO-dependent polycythemias are called primary familial and congenital polycythemias (PFCP). Some, but not all, are from gain of function of EPO receptor mutation.³⁴ In contrast, there are a growing number of high EPO congenital polycythemias, where patients may have normal levels of EPO, but they are inappropriately high for the levels of their hematocrits. In the same family there may be individuals with disease states who have a normal or high-normal hematocrit and EPO levels inappropriate for their levels of hemoglobin. They could be either autosomal recessive, such as Chuvash polycythemia,³⁵

or have other mutations of the von Hippel-Lindau gene,³⁶ or have an autosomal dominant trait. The biology of these disorders is not known.

PFCP was popularized by the case of an Olympic gold medalist, Eero Mäntyranta from Finland, who was almost stripped of his medals because he had very high hemoglobin levels and was accused of blood doping. His doctor found that other family members had similar physical attributes, such as the typical ruddy complexion. This eventually led to a study of the Mäntyranta family, and the disorder was uncovered.

Primary polycythemic disorders include acquired mutations of hematopoietic stem cells and PFCP. To distinguish between primary and secondary polycythemias it is useful to grow erythroid progenitors isolated from peripheral blood in culture. The erythroid progenitors are washed free of serum and then exposed to culture conditions with increasing EPO concentrations. The primary polycythemias are either hypersensitive, which occurs in PFCP, or as with PV, some erythroid progenitors grow in the absence of EPO.

PFCP is an autosomal dominant disorder characterized by very low EPO level. The EPO is as low or lower than observed in PV patients and in some cases it cannot be detected. PFCP patients have been frequently misdiagnosed with PV. PFCP patients have polyclonal hematopoiesis, which can be measured in females using X chromosome inactivation. Patients have normal white cells and platelet levels and normal cytogenetics. Sometimes they have splenomegaly, although this is not characteristic of the disease. PFCP does not usually transform to MDS or acute leukemia, although at least 1 patient who had heavy doses of chlorambucil and radiophosphorus developed MDS/acute leukemia.

The biology of PFCP is relatively clear. EPO binds its receptor, which dimerizes, resulting in the activation of the cytoplasmic domains by phosphorylation of the receptor and also a protein in the signal transduction cascade called STAT5. STAT5 activates the antiapoptotic protein BclX. STAT5 is a transcription factor, which translocates into the nucleus and activates many erythroid-specific genes. In some of the families with PFCP, there is a deletion of cytoplasmic domain. In these individuals, the signal is turned on as with the normal EPO receptor, but in this case the receptor remains active. The feedback mechanism to turn off the receptor occurs through the binding of a phosphatase, and as this region is absent, the signal cannot be turned off. These people have a normal initial signaling, but because the signal cannot be turned off, there is excessive proliferation with decreased apoptosis of erythroid progenitors.³⁴

A mouse model of this disease was created in which the mouse EPO receptor was replaced with a normal human EPO receptor as well as the mutated EPO receptor. The heterozygous animals are polycythemic and the homozygous animals are even more polycythemic. This is relevant not only to the study of polycythemia, but also to the study of EPO function. The role of EPO in the brain and other organs is just being determined. A growing body of evidence shows that ischemic myocardium could be markedly improved by EPO activity, and there are

more studies that indicate that the infarcted areas of the brain could be partially rescued by high EPO levels.^{37,38}

Increased EPO levels relieve hypoxic states, but the actual picture is more complicated. Inhabitants of high altitude, such as Quechua Indians in Peru, have high hemoglobin levels and develop polycythemia, but the Sherpas in Tibet and the high altitude dwellers in Ethiopia do not have high hemoglobin. This significant genetic variation in counteracting hypoxia with EPO in different populations has recently been confirmed.³⁹

The first and best known polycythemic states associated with increased EPO levels are those hemoglobin mutants that bind too tightly or have a high affinity to oxygen, resulting in decreased delivery of oxygen in the venous blood.⁴⁰ The body compensates by increasing its EPO levels, which elevate the hemoglobin level to deliver the optimum amount of oxygen. Multiple investigators have shown that EPO is regulated by hypoxia.⁴¹ Hypoxia-inducible factor alpha (HIF-1 alpha) binds to the hypoxia responsive element, which is downstream of the EPO gene.⁴² The activity of HIF-1 alpha is increased by a lowered oxygen tension.⁴³ The von Hippel-Lindau protein plays an important role in hypoxia sensing. It binds to the hydroxylated HIF-1 alpha and serves as a recognition site of an E3-ubiquitin ligase complex. In hypoxia, the nondegraded HIF-1 alpha forms a heterodimer with HIF-beta and leads to increased transcription of hypoxia-inducible genes, including EPO, and also glycolytic enzymes, some cell-cycle proteins, vascular endothelial growth factor (VEGF), VEGF receptors, and many other genes.^{44,45} Mutations in the von Hippel-Lindau gene can lead to polycythemia, which is endemic in Russia, and is the first known disorder of a congenital defect of hypoxia sensing.⁴⁶ Polyakova, a hematologist from Cheboksary, Chuvashia, described this disorder in 1974 as an autosomal recessive disease. These individuals have normal platelets, normal leukocytes, a normal p50, do not have splenomegaly, but they have either inappropriately normal or increased EPO levels. These individuals are of Asian ethnic origin, migrated to Russia about 800 years ago, live in the Chuvash Autonomous Republic, and about 10,000 of them have this mutation.⁴⁷⁻⁴⁹

Because this is an ethnically isolated population, it was relatively straightforward to find a family with more than 1 affected individual, and by the process of positional cloning, identify the chromosomal location and find the narrow chromosome markers and the defective gene, which turned out to be the von Hippel-Lindau gene.⁴⁷ The affected individuals are homozygous for this mutation, which is in contrast to von Hippel-Lindau syndrome, where a single allele of von Hippel-Lindau is mutated. If this mutation is inherited, a single cell develops cancer, such as renal cell cancer if the mutation occurs in the kidneys and cerebral hemangioma if the mutation is in the cerebellum. Chuvash polycythemia is a homozygous defect of the negative regulator of HIF-1 alpha, which leads to gain of function of hypoxia signaling, resulting in an upregulation of HIF-1 target gene. People with this defect have multiple abnormalities, one of which is high EPO levels. They also have varicose veins, strokes, and decreased blood pressure. Their complete phenotype is just being revealed.

The von Hippel-Lindau mutation is not an uncommon cause of congenital polycythemia with normal to increased EPO. Researchers in Belfast found 3 unrelated Indian, Pakistani, and Punjab families homozygous for the same defect.⁵⁰ Caucasians were either homozygous or double heterozygous for this defect, and there is at least 1 African American identified with this disorder. This mutation occurred once in evolution, multiple racial ethnic groups shared it, and it originated before the races split approximately 40,000 years ago.⁵¹ This suggests that the heterozygous individuals may have some survival advantage, but the survival advantage is speculative and remains to be identified.

Several families with PV were found to have acquired polycythemia, and more importantly, female patients had clonal hematopoiesis. A study of these families suggests that there may be multiple family members with PV, which would indicate that there is an inheritance of family predisposition. Study of these families is facilitated by rapidly evolving genetic techniques to uncover the defect that leads to PV and perhaps other MPD.

Idiopathic Myelofibrosis

IMF, also known as agnogenic myeloid metaplasia or myeloid metaplasia with myelofibrosis, has an incidence between 0.5 and 1.5 per 100,000 compared to PV or ET, which has an incidence of about 3 per 100,000. Because the median age at diagnosis of IMF is 65 years, any therapeutic modality for these patients has to take into account that they are elderly. The average survival reported is 3.5–5.5 years.^{52,53} IMF is a more aggressive disease than PV and ET, and 50% of patients die in about 4 years.^{54,55}

IMF, spent phase PV, and ET are marked by progressive anemia and hepatosplenomegaly, nucleated red cells, and teardrop forms in the peripheral blood. A bone marrow scan of an IMF patient shows reticulin sclerosis or mild to moderate collagen fibrosis accompanied by a lack of hematopoietic progenitor cells.^{56,57} The majority of patients with progressive anemia require transfusional support. Splenectomy is required for some patients due to either mechanical problems with a massive spleen or serious cytopenias.

Disruption of the Hemostatic Mechanisms in Myeloproliferative Disorders

Thrombosis and bleeding are frequent in MPD. The prevalence of thrombosis is about 20%–30% in PV, ET, and IMF, but less in CML. The prevalence of bleeding is about 20% in PV, CML, and ET, and 60% in IMF because of thrombocytopenia. In patients with PV, 44% die of thrombosis and <10% die of bleeding.^{58,59}

Coagulation reactions are initiated by tissue factor. Tissue factor, a transmembrane protein, plays a central role in the coagulation cascade. Tissue factor complexes with factor VII; factor X is converted to factor Xa and then factor Xa with factor V rapidly cleaves prothrombin to form thrombin.⁶⁰ Thrombin is generated in relatively small quantities in vivo, and this small amount of thrombin stimulates the formation of more thrombin by a positive feedback loop.⁶⁰ This reaction proceeds rapidly because of the thrombin activation of factor V Leiden and the supplementation of factor Xa via this intrinsic pathway so that ultimately fibrinogen is cleaved to fibrin to form a clot. In ad-

dition to thrombin's procoagulant action, which includes the activation of factor XIII to covalently crosslink fibrin, thrombin has anticoagulant properties, it binds to thrombomodulin altering its procoagulant properties, and now thrombin and thrombomodulin activate protein C. The protein C together with protein S limits coagulation.⁶¹

Endogenous anticoagulant mechanisms include the thrombomodulin, protein C, protein S complex; and antithrombin, which inactivates all of the serine proteases, tissue factor pathway inhibitor (TFPI), and annexin A5. All of these mechanisms that are part of the intrinsic system, the extrinsic system, and the anticoagulation system have chokepoints, such as deficiencies in TFPI, protein C, protein S, antithrombin, plasminogen, and plasminogen activator. Activated protein C resistance from factor V Leiden and thrombophilia could arise as a result of disruption of these chokepoints. Each step in the coagulation process has a counterbalance that inhibits that step. In addition to this mechanism for thrombophilia, there are gain of function mechanisms, ie, the polymorphism in which there is an increased expression of factor II and other conditions in which there are increases in factor VIII and factor IX and factor XI that are associated with an increased risk of venous thrombosis. Homocysteinemia is a thrombophilic condition, as are the autoimmune-mediated thrombosis syndromes of the antiphospholipid syndrome and thrombotic thrombocytopenic purpura.

Very little information is available as to how these mechanisms relate to MPD. Heterozygosity for polymorphism of factor V Leiden in PV and ET patients has a prevalence similar to that in the general population (14/304).⁶² However, a study from Denmark reported a higher incidence (7/50) in a single series of MPD patients.⁶³ A variety of changes in coagulation have been described: prolongation of aPTT; decreases in free protein S and protein C; decreases in coagulation factors II, VII, and X; an increase in anticardiolipin IgM levels; and an increase in soluble thrombomodulin. No differences in antithrombin, plasminogen, tPA, PAI-1, thrombin antithrombin complex, or D-dimer have been reported. Thus, there is no convincing independent association with thrombosis in the myeloproliferative conditions.

Two mechanisms are known to affect thrombosis: (1) the increased level of red blood cells in PV, and (2) the elevation in platelet count with ET. Newer findings include the roles of tissue factor and polymorphonuclear leukocytes in clotting, the platelet surface as a contributor to phospholipid-dependent coagulation reactions, and the entity of microparticles. Tissue factor is the central initiator of coagulation reactions, binding to factor VII to activate factor X. Tissue factor is present in the tunica media and adventitia of blood vessels. It is also synthesized by blood leukocytes, and this production may be a possible mechanism for thrombosis in MPD.⁶⁴ It has been shown recently that tissue factor circulates in blood in an inactive form and binds to form fibrin, thereby propagating thrombus formation.⁶⁵ Differences in circulating tissue factor may exist in MPD patients. Polymorphonuclear leukocytes and endothelial cells are activated and there is evidence for hypercoagulation in MPD. Polymorphonuclear leukocyte activation was shown to occur through increased

expression of CD11b, LAP antigen, elastase, both intracellular and soluble, and myeloperoxidase, whereas endothelial activation was measured by increased soluble thrombomodulin and von Willebrand factor.⁶⁶ However, no association was found for thrombosis, which the authors speculated might have been because of the relatively small size of the study.

In addition, increases in platelet microparticles (normal 5.2%, PV 12%, ET 11%, and IMF 11%), platelet neutrophil conjugates (normal 6.8%, ET 10.4%, and PV 8.3%), and platelet monocyte conjugates (normal 8.0%, ET 15.0%, and PV 15.4%) were found in MPD patients. However, there was no statistically significant correlation with venous or arterial thrombosis. There was a trend toward slightly more microparticles in patients with thrombotic histories, but none of these measurements reached statistical significance.⁶⁷

Cellular microparticles circulating in blood are plasma membrane fragments from stimulated and apoptotic cells. Platelet microparticles express phospholipids, have procoagulant activity, and can be generated by high shear stress, and may promote thrombosis or they may result from thrombosis. Phospholipid-dependent blood coagulation reactions include tissue factor VII activation of X, IXa, VIIIa activation of X, and factor X binding to phospholipid together with factor Va, generating thrombin.⁶⁸ These reactions may occur on the surfaces of activated platelets and on microparticles and may play a role in stimulating thrombosis. These are novel concepts for a thrombotic role for platelets in MPD. It is possible that platelets may have increased procoagulant activity via increased expression of phosphatidylserine.⁶⁸ A different approach examined DNA transcripts by microarray and by serial analysis of gene expression to determine novel mechanisms for thrombosis. This study, by working backwards to understand what mechanisms are operating, aimed to determine changes in gene expression that may identify patients who have a thrombotic tendency.⁶⁹

Hyperhomocysteinemia is a risk factor for thrombosis; it is widely prevalent in patients with MPD, 35% in controls compared to 56% in PV, 70% in ET, and 60% in IMF patients, indicating that there are increases in homocysteine levels in these groups. Mean homocysteine levels were determined to be 13.9 ± 4.2 $\mu\text{M/L}$ in PV, 12.8 ± 3.7 $\mu\text{M/L}$ in ET, and 11.3 ± 4.2 $\mu\text{M/L}$ in IMF compared to 9.7 ± 2.8 $\mu\text{M/L}$ in controls. Remarkably, all of these increases were related to nutritional deficiencies of B vitamins, vitamin B₁₂, and folic acid, and supplementation led to normalization in all of the cases.⁷⁰

Acquired von Willebrand syndrome is an established cause of bleeding in MPD. Data from an international registry on acquired von Willebrand syndrome indicate that MPD are the second most frequent clinical conditions associated with acquired von Willebrand syndrome, constituting about 12–15% of all patients with this form of von Willebrand disease.⁷¹ Acquired von Willebrand syndrome is also associated with other conditions, such as lymphoproliferative conditions, most prominently monoclonal gammopathy of unknown significance, other immunologic conditions, congenital and acquired cardiac defects, aortic stenosis, and other conditions.^{72,73} In MPD, about half of

patients have deficiencies in von Willebrand factor, and about half of the patients with acquired von Willebrand syndrome have bleeding problems. Half of the patients with acquired von Willebrand syndrome in MPD are bleeders with mildly prolonged bleeding time. The partial thromboplastin time is unaffected, factor VIII level is borderline to low, and the von Willebrand antigen tends to be normal. The ristocetin cofactor is low and tends to be about half of the von Willebrand factor antigen. Ninety percent of these patients are lacking high molecular weight multimers of von Willebrand factor. Acquired von Willebrand syndrome in the setting of MPD is an acquired type IIa von Willebrand disease.⁷⁴⁻⁷⁶ This condition can temporarily be treated with von Willebrand factor concentrates; however, the effect is not long lived. This condition is largely related to adsorption of von Willebrand factor onto platelets. Reducing the platelet count should be the major treatment aim.

Hydroxyurea Treatment and Leukemia

HU is currently a prominent therapeutic modality for Ph-MPD and is frequently employed for patients requiring myelosuppression. HU with intermittent phlebotomy is a highly efficacious treatment strategy in the management of PV for appropriate patients.³ HU is also a primary treatment used for hematologic conditions such as sickle cell disease.⁷⁷ In a trial conducted by the PVSG, HU reduced the risk of thromboses in patients with PV.⁷⁸ In another study, patients treated with HU compared to a cohort treated only with phlebotomy had fewer thrombotic complications.⁷⁹ Similarly, in a randomized, placebo-controlled trial, the incidence of thrombotic complications reported by patients treated with HU for essential thrombocytosis decreased from 24% to less than 4%, indicating that HU has a role in antithrombotic therapy.⁸⁰ While HU has been shown to be effective in reducing the risk of thrombosis, the question that needs definitive clarification is to what extent HU is safe for patients with Ph-MPD.

The role HU may play in leukemic conversion is not clear. Several nonrandomized studies have supported or refuted a significant increase in leukemic conversion with long-term use of HU. In essential thrombocytosis (ET), the rates of leukemic transformation following HU treatment reported in the literature range from 0 to 5.5%.^{81,82} In PV, the rates range from 2.1% to 10%.^{79,83} Data from the PVSG show the comparative incidence of acute leukemia by protocol and treatment. It is important to recall that on the pivotal PVSG 01 trial increased leukemic conversion with agents subsequently proven to be leukemic occurred after 7 years. In a trial of 795 weeks (15 years), which compared the incidence of a leukemic conversion in 51 patients treated only with HU to a historic cohort of patients treated with phlebotomy only, the trend for leukemic conversion was greater in patients treated with HU. It is important to note, however, that these differences did not meet statistical significance.⁷⁹

In a study reported by Sterkers et al,⁸⁴ a significant number of ET patients who developed either MDS or acute leukemia were found to have abnormalities of chromosome 17p; many of these patients were treated with HU alone. This paper concluded that acute leukemia in ET patients who were treated with HU alone

was therapy related. Several groups have shown that combination therapy with HU and alkylating agents increased the risk of leukemic conversion.⁸⁵⁻⁸⁸ A caveat of these observations proposes that if combination therapy is required to control platelet numbers, it is not the agents that are causing the transformation; rather it is the presence of a more aggressive disease, with a greater tendency to transform to acute leukemia.

Platelet-Lowering in MPD

The decision to use cytoreductive drugs to lower platelet numbers or to inhibit platelet function is often problematic for clinicians. The questions that remain include: (1) What are the pathophysiologies of bleeding and thrombosis in ET and PV? (2) Are they related to platelet number, platelet function, platelet turnover, or other yet to be defined factors? (3) What is the risk of an elevated platelet count in ET? (4) Is there clear evidence that elevated platelet levels are bad? (5) What are the risks in the symptomatic patient, in the asymptomatic patient with very high platelet counts, and in patients with mild to moderate thrombocytosis?

Therapy with low-dose aspirin in patients with thrombocytosis suppresses thromboxane biosynthesis by platelets, which is increased in patients with PV and ET. The ECLAP group from Italy studied the effect of low-dose aspirin in preventing thrombosis and found that control of microvascular symptoms in patients without a bleeding diathesis can be achieved. For example with erythromyalgia, which results from platelet aggregation, low-dose aspirin can prevent the pain caused by microvascular thrombosis.^{89,90}

Newer Agents for Thrombocytosis

Newer agents have been found to be therapeutically effective in the management of Ph-MPD.^{91,92} One of them, anagrelide, a cyclic AMP phosphodiesterase inhibitor, inhibits platelet aggregation in mice. It prevents megakaryocyte maturation in humans and thereby reduces circulating platelet numbers.⁹³ Long-term treatment with anagrelide was studied in an open-label multicenter international trial to determine anagrelide's long-term safety with respect to leukemogenesis and efficacy in patients with Ph-MPD (PV and ET).⁹⁴

In this safety trial of over 3,660 patients, 2,251 had ET, 462 had PV, and the rest had CML or other MPD. Almost three quarters of patients received previous myelosuppressive therapy; the majority of these patients were treated with HU. A small percentage of previously untreated ET and PV patients received only anagrelide as therapy. Of the ET group, only 47 patients transformed to acute leukemia within the time of the study (Table 2). It is important to note that when the cumulative mean dose of anagrelide was calculated for these patients, those who experienced leukemic conversion received less anagrelide than patients who did not convert. Typically when a drug is leukemogenic, patients in the transformed group receive higher doses than those who do not transform. These differences in the median cumulative doses reached statistical significance, which suggests that the leukemic transformation may be independent of the drug (see Table 2).⁹⁴ Similarly, in the group of 462 PV patients, 13 transformed to acute leukemia or myelo-

Table 2. Transformation of ET and PV Patients

Population	Transformed	Nontransformed	Median anagrelide cumulative dose	
	n	n	Transformed	Nontransformed
ET	47	2,204	342 mg*	746 mg*
PV	13	449	210 mg†	465 mg†

* $P=$.005.† $P=$.222.

ET=essential thrombocythemia; PV=polycythemia vera.

dysplastic syndrome. The vast majority of these patients did not transform, and the amount of drug received by those who did transform was lower than those who did not transform. While these results did not meet statistical significance, they suggest that transformation does not appear to be dose-related.⁹⁴

In this large cohort of ET and PV patients with a maximum follow-up of over 7 years, the conversion to acute leukemia for the ET group was 2.1% and for the PV group, 2.8%. All patients who converted had received prior cytoreductive therapy in addition to anagrelide. None of the converted patients had received anagrelide only. Of the patients treated with anagrelide for 3 years or more, none with ET and 0.26% with PV transformed. Further study will be necessary to confirm these initial findings on the safety of anagrelide for long-term use and its potential for leukemogenesis because, as already seen in the PVSG trial, more than 7 years was required for the leukemias to be observed.⁹⁵

Anagrelide is effective in reducing a mean platelet count of over 1,000,000/ μ L into a more acceptable level of about 450,000/ μ L in patients with ET and PV. It is not yet established that a reduction in platelets reduces the complications of hemorrhage and thrombosis in ET and PV. A cohort of over 1,600 patients who had a prior history of hemorrhage and thrombotic complications, mostly in the form of transient ischemic attacks, was observed for 3 years following treatment with anagrelide to reduce platelet numbers. This study showed that there was a corresponding decrease in reported symptoms with decreasing platelet counts.⁹⁶ However, during a mean duration of anagrelide treatment of 65 weeks, 17% of patients withdrew from studies because of adverse events or abnormal laboratory tests. The most frequently reported adverse events were headache, palpitations, diarrhea, edema, nausea, and other complaints. By starting anagrelide at a lower dosage, these side effects can be minimized.⁹⁷

Treatment of Idiopathic Myelofibrosis

According to the Italian registry for myelofibrosis, established in 1999,⁹⁸ 32% of patients do not receive any form of therapy, and of the remaining 68%, 45% receive therapy for anemia or thrombocytopenia, and 47% receive therapy for progressive splenomegaly. Excluding approximately 10% of patients who are candidates for allogeneic stem cell transplantation, the remaining approaches to therapy are conservative. In the last 2 years, there has been a move to disease-oriented therapy, which means using mechanism- or molecular-targeted therapies. There is rationale for using tyrosine kinase inhibitory therapy

Table 3. A Summary of Phase II Trials With Imatinib Mesylate in IMF

Patients	Initial dose	Anemia Response (%)	Splenomegaly Response (%)
23 ¹⁰⁰	400 mg	0	8.6
18 ¹⁰¹	400 mg	16	71
14 ¹⁵⁰	200 mg	16.6	0
11 ¹⁵¹	400 mg	27	NA
8 ¹⁵²	400 mg	0	37
13 ¹⁵³	600 mg	0	0
19 ¹⁵⁴	400 mg	0	45
106 Overall		7.5	27.3

in myelofibrosis because platelet-derived growth factor receptors (PDGF-R), vascular endothelial growth factor receptor-2 (VEGF-R2), and fibroblast growth factor (FGF) receptor have all been implicated with the pathogenesis of the disease.⁹⁹

Seven phase II trials (Table 3) have been carried out with imatinib mesylate in myelofibrosis; only 2 have been published as full-length papers and the others as abstracts.^{100,101} More than 100 patients were treated. Most studies used 400 mg as the initial dose, and as expected in phase II trials with small numbers of patients, the responses were quite different. The mean response rate with imatinib for anemia was no more than 7%, and for splenomegaly 27%. However, the trial from the University of Texas M. D. Anderson Cancer Center (MDACC) showed that there was a 71% response with splenomegaly.¹⁰¹ The toxicity with imatinib in myelofibrosis was high; up to 57% of the patients discontinued the drug for this reason. These studies indicated that imatinib is not an effective drug for myelofibrosis, as the only response seen was with splenomegaly. Imatinib is less well tolerated in patients with myelofibrosis than in patients with CML. This drug has additional side effects of frequent leukocytosis and thrombocytosis.

Receptor Tyrosine Kinase Inhibitors

SU5416, a small molecule that inhibits VEGF receptor tyrosine kinase, was used in 3 patients with myelofibrosis at MDACC. These 3 patients were treated as part of a phase II study in chronic MPD. Sugen has discontinued the drug because of disappointing results. Only 1/3 of patients had a partial response to this therapy, with high toxicity.¹⁰²

Farnesyltransferase Inhibitors

The rationale to use farnesyltransferase inhibitors depends on the proliferative activity of these enzymes on cells, particularly on myeloid cells. In vitro evidence shows that farnesyltransferase inhibitor reduces the proliferation of the myeloid clone (from myelofibrosis). Farnesyltransferases intervene in the posttranslational and modification of Ras protein.¹⁰³ Ras protein binds to the inner surface of the cell membrane, operates as a molecular switch from inactive to an active molecule, and is important in a series of pathways, particularly cytoskeletal organization, gene transcription, and cell proliferation.¹⁰⁴

Tipifarnib, a farnesyltransferase inhibitor, is the only agent that has activity in myelofibrosis. In a trial of 40 patients at MDACC,

Table 4. Transplant Experience in IMF Patients

n	Mean age	Poor risk	Regimen	TRM	CR	OS
55 ¹¹⁴	42	50%	Myeloablative	27%	40%	14% (>45 y) at 5 yr; 62% (<45 y)
25 ¹¹⁷	48	28%	Myeloablative	48%	33%	41% at 2 yr
56 ¹¹⁶	43	23%	Myeloablative	32%	53%	58% at 3 yr
4 ¹³¹	56	100%	Fludarabine-melphalan	0%	4/4	100%, 3 yr
20 ¹³²	54	100%	Nonmyeloablative	0% at d 100		90% at 18 mo

IMF=idiopathic myelofibrosis; TRM=treatment-related mortality; CR=complete response; OS=overall survival.

8 with myelofibrosis, 2/8 patients showed an improvement with respect to anemia and 4 had a decrease in splenomegaly. A preliminary account of a trial in which myelofibrosis patients were treated with lower doses of tipfarnib was reported at ASH 2003.¹⁰⁵ The response with respect to anemia was 5%, and 61% with respect to splenomegaly; 40% of patients had a clinically significant reduction in splenomegaly although toxicity was high. Tipfarnib or the farnesyltransferase inhibitor can be considered an experimental therapy, and is noteworthy because of the reported good response in splenomegaly.¹⁰⁶

Angiogenesis Inhibitors

There is solid evidence about angiogenesis in myelofibrosis. Microvessel grade in bone marrow of patients with myelofibrosis is higher than in other chronic MPD¹⁰⁶ and there is a correlation between the intensity of bone marrow angiogenesis and prognosis.^{107,108} Recent evidence indicates that the spleen in this disease is a site of neoangiogenesis.¹⁰⁹ New therapies targeting angiogenesis include the use of thalidomide. A pooled analysis was conducted of 5 phase II trials with thalidomide administered at standard doses (200–400 mg). The trials were from Europe and the United States with a total of 63 patients. In this analysis, 65% of the patients discontinued the drug due to side effects before the 6 months of planned treatment. Adverse events from thalidomide were reported in more than 90%. Evidence from efficacy analyses indicates that thalidomide provides good response with improvement of anemia (29.6%), improvement of thrombocytopenia (38.5%), and reduction of splenomegaly (36.7%) by more than 2 cm. This drug produced untoward hematological effects: an increase of white blood cells was observed in approximately 18% of the patients, or an increase in platelet counts to more than 500,000/ μ L, and in 1 case this myeloproliferative reaction was associated with pericardial extramedullary hematopoiesis.¹¹⁰ These results prompted a test of low-dose thalidomide. A trial in 21 patients treated with a combination of 50 mg of thalidomide and 30 mg of prednisone produced impressive responses. Improved anemia was seen in 62% of the patients. Of the 10 transfusion-dependent patients, 4 became transfusion independent, and improved platelet counts were seen in 6 patients. Only 19% of the patients showed a 50% reduction in spleen size. The lower dose of thalidomide in combination with prednisone reduced side effects and toxicity.¹¹¹

The European Collaboration on Myelofibrosis completed a phase II trial with 63 patients, including those with advanced myelofibrosis. Single-agent thalidomide (50 mg) was used as therapy along with current treatment regimens. Dose escalation to 400 mg was used in those who could tolerate it. The maximum tolerated dose was found to be 100 mg. The side effects were not negligible because 51% dropped out at 6 months of therapy. Side effects included constipation in 64%, increased fatigue in 50%, sleepiness, and neurological disturbances. In 16% of the patients an increase of platelet count to more than 500,000/ μ L was observed and in 1 case this reactive thrombocytosis was associated with deep vein thrombosis. Response to treatment showed improvement in anemia in 49% of the patients with a 27% reduction in transfusion dependency. White blood cell counts (500×10^9 /L) improved in 16% of patients and 38% showed a decrease in spleen size. Amelioration of anemia and thrombocytopenia and reduction of splenomegaly was achieved by 61% of the patients.¹¹² The findings from these 2 phase II trials with a total of 84 patients provide enough evidence to recommend thalidomide in myelofibrosis. Low-dose thalidomide is better tolerated than standard dose, and responses with low doses are similar to standard doses. Combination with prednisone seems to improve both response and tolerability.

Thalidomide may be used as first-line therapy for anemia and thrombocytopenia or may be used after failure with danazol. Thalidomide is still an experimental drug for myelofibrosis. The relatives of thalidomide, CC5013 and CC4047, inhibit neoangiogenesis and cytokine production and may also be active in this disease. Proteasome inhibitors may also produce a good response in myelofibrosis because they inhibit NF κ B transcriptional activity, which is activated by megakaryocytes in myelofibrosis. Inhibitors of VEGF or VEGF receptors also have a good rationale for use in myelofibrosis. One difficulty in testing new drugs is collecting enough patients in a short time. There is also the problem of prioritizing the number of novel drugs to test, and more importantly, comparing the results of these trials. From the number of European and American research consortia, phase III or phase II trials with comparative designs need to be planned.¹¹³

Bone Marrow Transplant: An Option for IMF

The transplant outcome for IMF patients is similar to that for CML patients, about 50% mortality after allogeneic stem cell transplantation with appropriate age groups. The transplant-related mortality for fully myeloablative approaches ranges from 27% to 32%.^{52,114–117} To improve the overall survival rate with fully ablative transplants in patients less than 65 years (Table 4), it has become necessary to identify patients with poor prognosis who could benefit from an allogeneic stem cell transplant. The Lille scoring system assists in determining transplant candidates.¹¹⁸ In this classification scheme, the presence of 2 adverse factors, such as hemoglobin of less than 10 g/dL and a white count of less than 4,000/ μ L or greater than 30,000/ μ L, places the patient in a high-risk category with an expected median survival of about a year. One of these adverse factors places the patient in an intermediate-risk category with a median survival of about 2 years. Thus, allogeneic transplant needs to be studied

further in order to select appropriate patient cohorts who are projected to have a poor outcome with medical management.

A retrospective study using data from 2 transplant studies¹¹⁹ has clearly shown that hematopoietic recovery was not a problem for patients with myelofibrosis. The probability of achieving a neutrophil recovery was >86% by 30 days and roughly 97% by day 50 after the transplant. Marrow fibrosis is not a risk factor with respect to graft failure. However, severe marrow fibrosis delays neutrophil recovery. The results for patients with severe marrow fibrosis indicate that by day 21 only 30% of the patients show neutrophil recovery, but in those with less severe marrow fibrosis, around 70% show neutrophil recovery. Marrow fibrosis is a reversible process. In most patients there was a complete resolution of marrow fibrosis at a median of 6 months after transplantation and in most cases during the first year posttransplant.¹²⁰

Using stem cells from the peripheral blood instead of bone marrow may improve the speed of neutrophil recovery. In a study comparing the source of stem cells from peripheral blood versus bone marrow, neutrophil recovery by 3 weeks posttransplant was roughly 80% using peripheral blood versus 50% from bone marrow, indicating that peripheral blood stem cells may be an option to consider for these patients. The other option may be splenectomy. Patients who have been splenectomized before transplantation have a higher mean probability of neutrophil recovery by day 21 (67%), than those who have not been splenectomized (36%).¹²¹ Splenomegaly is associated with an increased risk of graft failure because of sequestration of the stem cells from the graft. It can also be associated with relapse because of residual malignant cells in the spleen.¹²² Splenectomy, on the other hand, is associated with an increased risk of acute graft-versus-host disease, which has been described in CML patients.^{123,124} There could also be delayed or impaired immune recovery and severe bacterial infections. The operative mortality and morbidity are about 10% and 30% respectively; however, splenectomy is associated with a faster hematopoietic recovery.

Retrospective analysis shows that there is no difference in median transplant-related mortality 1 year following transplant between those who were splenectomized (30%) and those who were not (31%). The median 5-year event free survival is 49% for the nonsplenectomized patients and 35% for splenectomized patients, indicating that there is no clear significant difference.¹²¹ Splenectomy should not be recommended for all patients before allogeneic transplantation. It should be discussed for some patients where a faster neutrophil recovery is required and in those who have symptomatic splenomegaly before transplant or low transfusion efficacy that could jeopardize the early posttransplant period.

Transplant-related mortality (TRM) at 1 year is 25% when donor grafts come from HLA-identical siblings versus 60% when transplant is performed with alternative donors, but this data from the European Bone Marrow Transplant (EBMT) group is based on a small number of patients.¹¹⁴ Transfusion is another factor that affects TRM; it is about 40% for those who receive red blood cell transfusion before transplant and 10% for those

who do not. A TRM below 20% can be achieved with a careful selection of patients. Myelofibrosis is another factor that affects TRM. Patients who do not have severe myelofibrosis have lower TRM (15%) versus those with severe fibrosis (48%). The rates can be modified according to the type of myeloablative conditioning regimen used.¹²⁵ TRM is 40% with a combination of irradiation and cyclophosphamide, and 21% with busulfan and cyclophosphamide (BuCy).¹¹⁶ A lower TRM with BuCy combination probably occurs because of a lower incidence of grade 2–4 acute graft-versus-host disease. The event-free survival for patients who receive a transplant graft from an HLA-identical sibling without any manipulation of the graft is about 54% by 5 years posttransplant. From the EBMT experience, event-free survival is 27% for patients with grafts from unrelated donors and for patients who received T-cell depleted grafts there is no long-term survivor.^{126,127}

Factors that impact survival include hemoglobin levels and marrow fibrosis. A transplant patient with a hemoglobin level above 10 g/dL has an event-free survival at 5 years of 61% compared to 28% for those who have lower hemoglobin levels. Similar curves were observed from patients who receive transfusion versus those who did not require transfusion before transplantation. For those with severe marrow fibrosis, the 5-year event-free survival is about 30% and for those with less severe marrow fibrosis, 52%.^{120,121}

Long-term survival data are available for more than 10 years from the time of transplant, and it is apparent that surviving patients are cured. This goal is achieved with allogeneic transplant in this setting. Transplant failure incidence is about 25% for unmanipulated HLA-identical sibling transplants. Three risk factors have been identified as predictors of treatment failure; these are the age of the patient (>45 years), the presence of a cytogenetic abnormality before the transplant, and absence of grade 2–4 acute graft-versus-host disease. There is clearly a graft-versus-myelofibrosis effect. Donor lymphocyte infusion could induce a complete remission in patients who relapse and whose marrow fibrosis has disappeared. The graft-versus-myelofibrosis effect could be the basis for new approaches to transplant therapy.¹²⁸⁻¹³⁰

The median age at diagnosis of patients with myelofibrosis is 65 years, so most patients are not eligible for a conventional transplant. Another transplant approach that may be more appropriate for older patients is to use a reduced-intensity conditioning regimen; in 1 small trial, 4/4 patients were still alive at 3 years (Table 4).¹³¹ The graft-versus-myelofibrosis effect is the basis for the reduced-intensity regimen for transplants. The MPD research consortium examined the role of a reduced-intensity regimen using a variety of induction therapies.¹³² For 20 IMF patients, with a median age of 54 years and an overall survival of 90% at 18 months' median follow-up, transplant-related mortality was 0% by day 100 posttransplant. IMF patients had their bone marrows stained for reticulin prior to and after transplant. Prior to transplant their marrows show typical myelofibrosis with a lack of hematopoietic progenitors. One year from the reduced-intensity allogeneic transplant, staining showed some collagen fibers and an abundance of normal hematopoi-

etic progenitors. A study of 20 patients by the EBMT demonstrated that nonmyeloablative transplants had no increased risk of graft failure with HLA-identical siblings. There is insufficient data to draw conclusions for unrelated donor transplants, and the follow-up period of the study is short. Patients achieved full donor chimerism in most cases and complete histohematological responses were observed; that is, the marrow fibrosis disappeared posttransplant with improvement in hematopoiesis. The 1-year TRM was less than 20% and event-free survival greater than 70%.¹³³ For patients in accelerated phase or blast phase, the results were not as good.

Eligibility for transplant should be based on the patient's life expectancy with standard treatments. For intermediate- or high-risk patients according to the GPS score, transplant can be considered as soon as the patient is ready for the procedure. If the patient is <50 years, a myeloablative conditioning approach is probably the best option. If the patient is >50 years, then a reduced-intensity conditioning regimen would be more appropriate. If a low-risk patient shows one of the signs or symptoms that are predictors of worse outcome—a drop in the hemoglobin levels to <10 g/dL, cytogenetic abnormalities, the need for red blood cell transfusion, or the presence of blast phase—then the patient should be transplanted.

Transplant experience is limited with unrelated donor transplants. It could be proposed for patients <40 years old. They could be transplanted with fully HLA-matched unrelated donors using high resolution typing, and be treated with a myeloablative regimen. If the patients have high- or intermediate-risk disease or if they have not been heavily transfused, they could be treated with a nonmyeloablative regimen. Using these criteria, the results are similar to those obtained with HLA-identical siblings.¹¹⁶ Peripheral blood might be the best stem cell source to use in these patients since the risk of graft failure is somewhat increased as compared to the HLA-identical sibling transplants.

Operative Approaches to Patients With Budd-Chiari Syndrome

Budd-Chiari syndrome (BCS) occurs in MPD patients and most frequently in young women.¹³⁴ Surgical approaches to the management of Budd-Chiari are therefore relevant to the management of patients with MPD. BCS is a liver-related condition associated with macrovascular or large vessel venous outflow disease. It can be associated with inferior vena cava thrombosis and occasionally concomitant portal vein thrombosis. The medical community has become more adept at making the diagnosis; this can be seen in an analysis of the types of presenting symptoms before 1990 and post 1990.¹³⁵ After 1990 there was a drop off of ascites, hepatosplenomegaly, abdominal pain, and gastrointestinal (GI) bleeding, and 20% of the patients that were diagnosed had no symptoms. The factors associated with BCS are similar to the different types of thrombophilias associated with thrombosis. The hematologic factors associated with BCS are thrombocytosis, polycythemia, and paroxysmal nocturnal hemoglobinuria; the coagulation factors include increased fibrinogen, increased von Willebrand factor, mutant factor V Leiden – G1691A, mutant prothrombin (G20210A),

decreased anti-thrombin III, decreased protein C, decreased protein S, and increased plasminogen activator inhibitor; the humoral factors include lupus anticoagulant, anticardiolipin antibodies, and hyperhomocysteinemia.¹³⁶ When patients are referred, they usually have already had a specific defect identified. If not, they are identified in order to help make a postoperative decision about anticoagulation.

Ultrasonography is done to identify portal vein patency. In addition to the standard CT scan or magnetic resonance imaging, BCS patients may need to undergo an invasive angiographic visualization and determination of the hemodynamics of the liver. The gradient may be present in the vena cava primarily because the liver is large, and this extrinsically compresses the intrahepatic inferior vena cava. The determination of gradients will help to determine the type of procedure required.

The histology of liver biopsies reveals the presence of acute or chronic changes as well as the degree of liver cirrhosis. Acute changes include centrilobular congestion, perivenular hemorrhage, hepatocyte atrophy, and sinusoidal dilatation, whereas chronic changes include perivenular fibrosis, cirrhosis, which is central-to-central, and variable nodular hyperplasia. These changes help to identify patients who should receive a transplant and those who need shunting. The advent of transjugular intrahepatic portosystemic shunts (TIPS)¹³⁷ has aided in the management of patients with chronic BCS and established cirrhosis, especially those patients with acute GI bleeding. TIPS provide an outflow for the portal vein through the metallic stent that is placed through the liver parenchyma, allowing flow into the atrium. This markedly decreases the risk for GI bleeds and ascites formation. However, TIPS are usually placed through the remnant of the hepatic vein. In cases where there is no hepatic vein, shunt placement has been difficult.

A new technique where the vena cava is cannulated through a femoral vein with an ultrasound probe can be used in patients who have no hepatic veins, no identifiable macroscopic outflow from the liver.¹³⁸ The transfemoral venous ultrasound probe is able to image the portal vein. Through a jugular approach, the TIPS needle stylet is placed through the anterior vena cava wall directly to puncture the portal vein. A PTFE- (polytetrafluoroethylene) coated TIPS is then inserted.¹³⁹ This very useful technique may be applicable more broadly in patients with BCS.

A number of different surgical shunts have been tested. In a side-to-side portocaval shunt (SSPCS), a small-diameter anastomosis (5–7 mm) is made between the vena cava and the portal vein. This can also be functionally achieved with an interposition graft, which is made out of PTFE or woven fabric. A mesocaval shunt is where the superior mesenteric vein is attached or drained into the vena cava usually using a synthetic interposition graft. A mesoatrial shunt is where the mesenteric vein is then drained into the atrium, again using a synthetic graft. Finally, there is a fourth type of surgical shunt, which is a modification combining a mesocaval shunt with a cavoatrial shunt. There is also a hybrid surgical and radiological technique, in which the compressed intrahepatic vena cava is stented open and an SSPCS is concomitantly performed. A Palmaz stent

Table 5. Outcome With Surgical Shunts in Budd-Chiari Syndrome

Procedure	Date	Patients	Survival, %	Follow-up, mo
PCS ¹⁴³	1992	20	90	98
PCS ¹⁴⁴	1998	16	81	67
MCS ¹⁴⁵	1990	11	73	43
MCS ¹⁴⁶	1991	18	94	66
MAS ¹⁴⁷	2000	13	77	42
MAS ¹⁴⁸	2000	8	38	17
PC/CAS ¹⁴⁸	2000	10	100	9

PCS=portocaval shunt; MCS=mesocaval shunt; MAS=mesoatrial shunt; PC/CAS=portocaval/cavoatrial shunt.

with an 18 mm balloon is placed to open up and dilate the intrahepatic vena cava to reduce the pressure to 5–7 mm Hg. The subsequent SSPCS then decompresses the mesenteric system. This procedure has a mean patency of about 15 months.¹⁴⁰

Lastly, liver transplantation itself is available for patients who have irreversible chronic liver disease, ascites, encephalopathy, portal hypertension, and established cirrhosis with little prospect of reversibility. Patients who have intrinsic thrombophilias due to coagulation defects, mutations in factor II and V, can be corrected by the use of liver transplantation, as it corrects the underlying thrombophilic defect.

Outcomes of Surgical Approaches in Budd-Chiari Syndrome

TIPS is useful derivative therapy for addressing the acute problem usually in those patients with acute GI bleeding who can not be controlled by medical therapy. However, the long term is problematic; as in the use of TIPS for other portal hypertensive situations, it is associated with stenosis of the TIPS shunt. In a study of 13 patients who failed medical therapy and underwent TIPS with a median follow-up of 3.2 ± 2.2 years, there were only 3 TIPS without dysfunction. There was 1 death, 1 patient was revised to SSPCS, and 1 who underwent a liver transplant. Improvement in ascites (from 12/13 pre-TIPS to 1/13 post-TIPS) with a Child-Turcotte-Pugh (CTP) score (from CTP score of 9 to CTP score of 6) was seen, with transaminases, and with reduction in portal pressure gradient (24 mm Hg pre-TIPS to 10 mm Hg post-TIPS).¹⁴¹ As these patient studies have shown, while improvements of ascites control and portal gradients can be acutely managed, the incidence of TIPS stenosis increases after about 3–6 months; they either fail or require subsequent modification.¹⁴²

Surgical shunts are classified according to the type of procedure: PCS is a portocaval shunt, MCS is a mesocaval shunt, MAS is a mesoatrial shunt, and there is also a combined portocaval caval atrial shunt, which has had variable patency rates. The portocaval shunt is considered to have the best patency because it is a fairly short graft. Two studies with 20 and 16 patients have shown survival of 90% and 81% with follow-up of 98 months and 67 months, respectively.^{143,144} Similarly, 2 studies with the MCS shunt were reported with 11 and 18 patients who were followed for 43 months and 66 months and showed survival of

73% and 94% respectively.^{145,146} The MAS was also reported in 2 studies with 13 and 8 patients who were followed for 42 and 17 months, and showed a survival of 77% and 38% respectively.^{147,148} Finally, the portocaval shunt followed by the caval atrial shunt was reported in 1 study with 10 patients and showed 100% survival with a follow-up of 9 months.¹⁴⁸ For optimum patency, the PCS should be considered as first choice, followed by the MCS, then the MAS, and lastly by the portocaval caval atrial shunts, which because of the longer length of the shunt, has a much reduced long-term patency rate (Table 5). Liver transplantation has a well-established survival rate in patients with BCS. The European liver transplant registry reports a 10-year survival rate of 63%. There were 391 patients observed from 1988 to 2001.¹⁴⁹ To ascertain the best treatment options for BCS, all surgical and interventional studies were put into context with medical management. Of 54 patients with BCS, 7 were medically treated, 2 underwent TIPS, and 43 had surgical shunts implanted, 24 MCS and 19 MAS. The MCS had a better patency rate than the MAS. Secondary revision was performed to improve shunt patency. All TIPS required revision. Eventually 6 of the 54 patients underwent liver transplantation with a 1-year survival rate of 83%, 5-year survival rate of 73%, and 5 were still alive with a follow-up of 2 to 9 years.¹⁴⁹

One issue surrounding the management of patients with pre-malignant conditions or MPD is how many of these patients develop cancers or leukemias and how they can be managed with coagulopathies or thrombophilias. In most studies, and there are very few of them, the risk for degeneration does not appear to be any greater than in patients who do not undergo liver transplantation. For example, in a report with 17 patients who received liver transplantation for BCS, 12 with MPD, 3 had mutations of coagulation factors and 2 had idiopathic BCS. MPD were managed with hydroxyurea and aspirin. The intrinsic coagulation defects like factors II and V do not require treatment because liver transplantation corrects the defect, and in types of BCS where the etiology cannot be determined, warfarin has been used to correct the hypercoagulable state.¹³⁴

A retrospective analysis from 1974 to 2002 of patients from the University of Pittsburgh was conducted, with 63 liver transplants performed in 50 people. With a mean follow-up of approximately 9 years, 2 patients were lost to follow-up. The patients transplanted were relatively young with more females than males and one-fifth of them had undergone previous shunt procedures. Only 32% of these were hematologic disorders. Forty-eight percent were labeled as “idiopathic,” but it should be recognized that many of these patients were transplanted before genetic testing was available for deficiencies in factors II and V as well as before the identification of other thrombophilic disorders. Following liver transplant, the postoperative anticoagulation therapy used was dextran with or without perioperative intravenous heparin followed by long-term maintenance oral coumadin. The 10-year survival rates are about 60%, which is consistent with the European liver registry data. Early mortality of less than 3 months was from fungal and bacterial sepsis, which is the most common cause of early mortality in most liver transplant series. Late mortality of

more than 3 months was from recurrent BCS in 40% of these late deaths. Nine patients were retransplanted due to graft dysfunction, early graft failure, recurrent BCS, and hepatic artery thrombosis (Fung et al, unpublished data).

Early recognition can minimize the progression of liver-related diseases, which makes available many more treatment options. The etiology is helpful in determining what procedure should be done as well as what type of anticoagulation is necessary either for surgical approaches or for transplant. All medical, surgical, and radiographic options should be considered in the treatment and management of BCS. Radiographic stents require monitoring because they have a high risk for stenosis or thrombosis. Ultrasonography of the radiographically placed stent is recommended every 6 months. Liver transplantation should be considered when there is evidence of impending liver failure or when complications are refractory in spite of medical management and stenting with or without radiographic stents. Because risk of disease recurrence is high, long-term anticoagulation therapy is recommended, unless there is convincing evidence that the hypercoagulable state is corrected solely by liver transplantation. Lastly, in this experience, the concern that MPD patients would have a high risk of developing acute leukemia has not appeared to be the case.

Conclusion

The chronic MPD—PV, IMF, and ET—are usually clonal, but more recently polyclonal hematopoiesis has been described. They can involve a multipotent or committed hematopoietic progenitor cell. They are heterogeneous with respect to their molecular, laboratory, and clinical features. Molecular markers such as c-Mpl and PRV-1 together with conventional analyses of karyotype with molecular techniques of gene expression profiling should prove useful for subclassifying these disorders and for risk stratification. As the biology of these diseases is constantly being revealed, there are newer agents for medical management of these patients. Transplant is an option where medical management has failed and the patient is eligible for transplant, and for those with complications of BCS there are surgical options available.

References

- 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.
- Wasserman LR. The management of polycythemia vera. *Br J Haematol*. 1971;21:371-376.
- 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.
- Zwicky C, Theiler L, Zbaren K, et al. The predictive value of clonogenic stem cell assays for the diagnosis of polycythemia vera. *Br J Haematol*. 2002;117:598-604.
- Cashman J, Henkelman D, Humphries K, et al. Individual BFU-E in polycythemia vera produce both erythropoietin dependent and independent progeny. *Blood*. 1983;61:876-884.
- Kanfer E, Price CM, Colman SM, et al. Erythropoietin-independent colony growth in polycythemia vera is not restricted to progenitor cells with trisomy of chromosome 8. *Br J Haematol*. 1992;82:773-774.
- Oehler L, Jaeger E, Eser A, et al. Imatinib mesylate inhibits autonomous erythropoiesis in patients with polycythemia vera in vitro. *Blood*. 2003;102:2240-2242.
- Spivak JL, Silver RT. Imatinib mesylate in polycythemia vera. *Blood*. 2004;103:3241-3242.
- Ferraris AM, Mangerini R, Racchi O, et al. Heterogeneity of clonal development in

- chronic myeloproliferative disorders. *Am J Hematol*. 1999;60:158-160.
- Asimakopoulos FA, Holloway TL, Nacheva EP, et al. Detection of chromosome 20q deletions in bone marrow metaphases but not peripheral blood granulocytes in patients with myeloproliferative disorders or myelodysplastic syndromes. *Blood*. 1996;87:1561-1570.
- Asimakopoulos FA, Green AR. Deletions of chromosome 20q and the pathogenesis of myeloproliferative disorders. *Br J Haematol*. 1996;95:219-226.
- Westwood NB, Gruszka-Westwood AM, Pearson CE, et al. The incidences of trisomy 8, trisomy 9 and D20S108 deletion in polycythemia vera: an analysis of blood granulocytes using interphase fluorescence in situ hybridization. *Br J Haematol*. 2000;110:839-846.
- Molitero AR, Hankins WD, Spivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. *N Engl J Med* 1998 Feb 26;338(9):572-80. 1998;338:572-580.
- Molitero AR, Siebel KE, Sun AY, et al. A novel thrombopoietin signaling defect in polycythemia vera platelets. *Stem Cells*. 1998;16:185-192.
- Horikawa Y, Matsumura I, Hashimoto K, et al. Markedly reduced expression of platelet c-mpl receptor in essential thrombocythemia. *Blood*. 1997;90:4031-4038.
- Li J, Xia Y, Kuter DJ. The platelet thrombopoietin receptor number and function are markedly decreased in patients with essential thrombocythemia. *Br J Haematol*. 2000;111:943-953.
- Harrison CN, Gale RE, Pezella F, et al. Platelet c-mpl expression is dysregulated in patients with essential thrombocythemia but this is not of diagnostic value. *Br J Haematol*. 1999;107:139-147.
- 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.
- Temerinac S, Klippel S, Strunck E, et al. Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. *Blood*. 2000;95:2569-2576.
- Najfeld V, Fuchs S, Merando P, et al. Fluorescence in situ hybridization analysis of the PRV-1 gene in polycythemia vera: implications for its role in diagnosis and pathogenesis. *Exp Hematol*. 2003;31:118-121.
- Bock O, Serinsoz E, Neusch M, et al. The polycythemia rubra vera-1 gene is constitutively expressed by bone marrow cells and does not discriminate polycythemia vera from reactive and other chronic myeloproliferative disorders. *Br J Haematol*. 2003;123:472-474.
- Pahl HL. PRV-1 mRNA expression and other molecular markers in polycythemia rubra vera. *Curr Hematol Rep*. 2003;2:231-236.
- Klippel S, Strunck E, Temerinac S, et al. Quantification of PRV-1 mRNA distinguishes polycythemia vera from secondary erythrocytosis. *Blood*. 2003;102:3569-3574.
- Liu E, Jelinek J, Pastore YD, et al. Discrimination of polycythemia and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-E response to erythropoietin. *Blood*. 2003;101:3294-3301.
- Goertler P, Doerner E, Johansson PL, et al. Thrombotic Complications in Four Subpopulations of Patients with ET Defined by c-Mpl Protein Expression and PRV-1 mRNA Levels. *Blood*. 2003;102:918a (abstr).
- Griesshammer M, Klippel S, Mohr U, Strunck E, Heimpe H, Pahl HL. PRV-1 mRNA Expression Discriminates Two Types of Essential Thrombocythemia and Can Predict Transition to Polycythemia Vera. *Blood*. 2003;102:659a (abstr).
- Perez-Encinas M, Bello JL, Perez-Crespo S, et al. Familial myeloproliferative syndrome. *Am J Hematol*. 1994;46:225-229.
- Turhan AG, Cashman JD, Eaves CJ, et al. Variable expression of features of normal and neoplastic stem cells in patients with thrombocytosis. *Br J Haematol*. 1992;82:50-57.
- Prchal JF, Prchal JT. Molecular basis for polycythemia. *Curr Opin Hematol*. 1999;6:100-109.
- Shih LY, Huang JY, Lee CT. Insulin-like growth factor I plays a role in regulating erythropoiesis in patients with end-stage renal disease and erythrocytosis. *J Am Soc Nephrol*. 1999;10:315-322.
- Prchal JT. Pathogenetic mechanisms of polycythemia vera and congenital polycythemic disorders. *Semin Hematol*. 2001;38:10-20.
- Friman S, Nyberg G, Blohme I. Erythrocytosis after renal transplantation; treatment by removal of the native kidneys. *Nephrol Dial Transplant*. 1990;5:969-973.
- Kralovics R, Prchal JT. Congenital and inherited polycythemia. *Curr Opin Pediatr*. 2000;12:29-34.
- Kralovics R, Indrak K, Stopka T, et al. Two new EPO receptor mutations: truncated EPO receptors are most frequently associated with primary familial and congenital polycythemia. *Blood*. 1997;90:2057-2061.
- Percy MJ, McMullin MF, Jowitz SN, et al. Chuvash-type congenital polycythemia in 4 families of Asian and Western European ancestry. *Blood*. 2003;102:1097-1099.
- Pastore YD, Jelinek J, Ang S, et al. Mutations in the VHL gene in sporadic apparently congenital polycythemia. *Blood*. 2003;101:1591-1595.
- Villa P, Bigini P, Mennini T, et al. Erythropoietin selectively attenuates cytokine production and inflammation in cerebral ischemia by targeting neuronal apoptosis. *J Exp*

- Med. 2003;198:971-975.
38. Prass K, Scharff A, Ruscher K, et al. Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. *Stroke*. 2003;34:1981-1986.
 39. Hopfl G, Ogunshola O, Gassmann M. Hypoxia and high altitude. The molecular response. *Adv Exp Med Biol*. 2003;543:89-115.
 40. Lichtman MA, Murphy MS, Adamson JW. Detection of mutant hemoglobins with altered affinity for oxygen. A simplified technique. *Ann Intern Med*. 1976;84:517-520.
 41. Bracken CP, Whitelaw ML, Peet DJ. The hypoxia-inducible factors: key transcriptional regulators of hypoxic responses. *Cell Mol Life Sci*. 2003;60:1376-1393.
 42. Srinivas V, Zhu X, Salceda S, et al. Hypoxia-inducible factor 1alpha (HIF-1alpha) is a non-heme iron protein. Implications for oxygen sensing. *J Biol Chem*. 1998;273:18019-18022.
 43. Heidbreder M, Frohlich F, Jöhren O, et al. Hypoxia rapidly activates HIF-3alpha mRNA expression. *FASEB J*. 2003;17:1541-1543.
 44. Bracken CP, Whitelaw ML, Peet DJ. The hypoxia-inducible factors: key transcriptional regulators of hypoxic responses. *Cell Mol Life Sci*. 2003;60:1376-1393.
 45. Meissner U, Allabauer I, Repp R, et al. Inducible expression of hypoxia-inducible factor 1alpha (HIF-1alpha) as a tool for studying HIF-1alpha-dependent gene regulation during normoxia in vitro. *Pharmacology*. 2003;69:74-78.
 46. Pastore Y, Jedlickova K, Guan Y, et al. Mutations of von Hippel-Lindau tumor-suppressor gene and congenital polycythemia. *Am J Hum Genet*. 2003;73:412-419.
 47. Gordeuk VR, Sergueeva AI, Miasnikova GY, et al. Congenital disorder of oxygen-sensing: association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and vascular abnormalities but not tumors. *Blood*. 2004;(Epub ahead of print).
 48. Percy MJ, Beard ME, Carter C, et al. Erythrocytosis and the Chuvash von Hippel-Lindau mutation. *Br J Haematol*. 2003;123:371-372.
 49. Ang SO, Chen H, Hirota K, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat Genet*. 2002;32:614-621.
 50. Percy MJ, Mooney SM, McMullin MF, et al. A common polymorphism in the oxygen-dependent degradation (ODD) domain of hypoxia inducible factor-1alpha (HIF-1alpha) does not impair Pro-564 hydroxylation. *Mol Cancer*. 2003;2:31.
 51. Liu E, Percy MJ, Amos C, et al. The Worldwide Distribution of the VHL 598C>T Mutation Indicates a Single Founding Mutation. *Blood*. 2003;102:204a (abstr).
 52. Fruchtmann SM. Transplant decision-making strategies in the myeloproliferative disorders. *Semin Hematol*. 2003;40:30-33.
 53. Barosi G. Myelofibrosis with myeloid metaplasia. *Hematol Oncol Clin North Am*. 2003;17:1211-1226.
 54. Rozman C, Giralt M, Feliu E, et al. Life expectancy of patients with chronic non-leukemic myeloproliferative disorders. *Cancer*. 1991;67:2658-2663.
 55. Kvasnicka HM, Thiele J, Werdn C, et al. Prognostic factors in idiopathic (primary) osteomyelofibrosis. *Cancer*. 1997;80:708-719.
 56. Thiele J, Kuemmel T, Sander C, et al. Ultrastructure of bone marrow tissue in so-called primary (idiopathic) myelofibrosis-osteomyelofibrosis (agnogenic myeloid metaplasia). I. Abnormalities of megakaryopoiesis and thrombocytes. *J Submicrosc Cytol Pathol*. 1991;23:93-107.
 57. Thiele J, Schmidt J, Sander C, et al. Ultrastructure of bone marrow tissue in so-called primary (idiopathic) myelofibrosis-osteomyelofibrosis (agnogenic myeloid metaplasia). II. The myeloid stroma (hematopoietic microenvironment). *J Submicrosc Cytol Pathol*. 1991;23:109-121.
 58. Brodmann S, Passweg JR, Gratwohl A, et al. Myeloproliferative disorders: complications, survival and causes of death. *Ann Hematol*. 2000;79:312-318.
 59. Wehmeier A, Daum I, Jamin H, et al. Incidence and clinical risk factors for bleeding and thrombotic complications in myeloproliferative disorders. A retrospective analysis of 260 patients. *Ann Hematol*. 1991;63:101-106.
 60. Furie B, Furie BC. Molecular basis of blood coagulation. In: Hoffman R, Benz Jr EJ, Shattil SJ, et al., eds. *Hematology Basic Principles and Practice*. New York: Churchill Livingstone; 2004:1783-1840.
 61. Dahlback B, Villoutreix BO. Molecular recognition in the protein C anticoagulant pathway. *J Thromb Haemost*. 2003;1:1525-1534.
 62. Ruggeri M, Gisslinger H, Tosi A, et al. Factor V Leiden mutation carriership and venous thromboembolism in polycythemia vera and essential thrombocythemia. *Am J Hematol*. 2002;71:1-6.
 63. Jensen MK, de Nully BP, Thorsen S, et al. Frequent occurrence of antithrombin antibodies, Factor V Leiden mutation, and perturbed endothelial function in chronic myeloproliferative disorders. *Am J Hematol*. 2002;69:185-191.
 64. Fleck RA, Rao LV, Rapaport SI, et al. Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. *Thromb Res*. 1990;59:421-437.
 65. Hathcock J. Vascular biology—the role of tissue factor. *Semin Hematol*. 2004;41:30-34.
 66. Falanga A, Marchetti M, Evangelista V, et al. Polymorphonuclear leukocyte activation and hemostasis in patients with essential thrombocythemia and polycythemia vera. *Blood*. 2000;96:4261-4266.
 67. Villmow T, Kemkes-Matthes B, Matzdorff AC. Markers of platelet activation and platelet-leukocyte interaction in patients with myeloproliferative syndromes. *Thromb Res*. 2002;108:139-145.
 68. Presseizen K, Friedman Z, Shapiro H, et al. Phosphatidylserine expression on the platelet membrane of patients with myeloproliferative disorders and its effect on platelet-dependent thrombin formation. *Clin Appl Thromb Hemost*. 2002;8:33-39.
 69. Gnatenko DV, Dunn JJ, McCorkle SR, et al. Transcript profiling of human platelets using microarray and serial analysis of gene expression. *Blood*. 2003;101:2285-2293.
 70. Fauschou M, Nielsen OJ, Jensen MK, et al. High prevalence of hyperhomocysteinemia due to marginal deficiency of cobalamin or folate in chronic myeloproliferative disorders. *Am J Hematol*. 2000;65:136-140.
 71. Federici AB, Rand JH, Bucciarelli P, et al. Acquired von Willebrand syndrome: data from an international registry. *Thromb Haemost*. 2000;84:345-349.
 72. Federici AB, Budde U, Rand JH. Acquired von Willebrand syndrome 2004: International Registry—diagnosis and management from online to bedside. *Hamostaseologie*. 2004;24:50-55.
 73. Vincentelli A, Susen S, Le Tourneau T, et al. Acquired von Willebrand syndrome in aortic stenosis. *N Engl J Med*. 2003;349:343-349.
 74. Raman BK, Sawdyk M, Saeed SM. Essential thrombocythemia with acquired von Willebrand's disease. *Am J Clin Pathol*. 1987;88:102-106.
 75. Friedenberg WR, Roberts RC, David DE. Relationship of thrombohemorrhagic complications to endothelial cell function in patients with chronic myeloproliferative disorders. *Am J Hematol*. 1992;40:283-289.
 76. Landolfi R. Bleeding and thrombosis in myeloproliferative disorders. *Curr Opin Hematol*. 1998;5:327-331.
 77. Steinberg MH, Barton F, Castro O, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. *JAMA*. 2003;289:1645-1651.
 78. Kaplan ME, Mack K, Goldberg JD, et al. Long-term management of polycythemia vera with hydroxyurea: a progress report. *Semin Hematol*. 1986;23:167-171.
 79. Fruchtmann SM, Mack K, Kaplan ME, et al. From efficacy to safety: a Polycythemia Vera Study group report on hydroxyurea in patients with polycythemia vera. *Semin Hematol*. 1997;34:17-23.
 80. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. *N Engl J Med*. 1995;332:1132-1136.
 81. Finazzi G, Barbui T. Efficacy and safety of hydroxyurea in patients with essential thrombocythemia. *Pathol Biol (Paris)*. 2001;49:167-169.
 82. Randi ML, Fabris F, Girolami A. Leukemia and myelodysplasia in patients with essential thrombocythemia treated with cytotoxic agents. *Haematologica*. 1999;84:1049-1050.
 83. Nand S, Stock W, Godwin J, et al. Leukemogenic risk of hydroxyurea therapy in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Am J Hematol*. 1996;52:42-46.
 84. Sterkers Y, Preudhomme C, Lai JL, 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.
 85. Murphy S. Therapeutic dilemmas: balancing the risks of bleeding, thrombosis, and leukemic transformation in myeloproliferative disorders (MPD). *Thromb Haemost*. 1997;78:622-626.
 86. Najean Y, Rain JD. Treatment of polycythemia vera: the use of hydroxyurea and piprobroman in 292 patients under the age of 65 years. *Blood*. 1997;90:3370-3377.
 87. Finazzi G, Ruggeri M, Rodeghiero F, et al. Second malignancies in patients with essential thrombocythemia treated with busulfan and hydroxyurea: long-term follow-up of a randomized clinical trial. *Br J Haematol*. 2000;110:577-583.
 88. Nielsen I, Hasselbalch HC. Acute leukemia and myelodysplasia in patients with a Philadelphia chromosome negative chronic myeloproliferative disorder treated with hydroxyurea alone or with hydroxyurea after busulfan. *Am J Hematol*. 2003;74:26-31.
 89. Landolfi R, Patrono C. Aspirin in polycythemia vera and essential thrombocythemia: current facts and perspectives. *Leuk Lymphoma*. 1996;22 Suppl 1:83-86.
 90. Landolfi R, Marchioli R, Kutti J, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. *N Engl J Med*. 2004;350:114-124.
 91. Gilbert HS. Modern treatment strategies in polycythemia vera. *Semin Hematol*. 2003;40:26-29.
 92. Barbui T, Finazzi G. Treatment indications and choice of a platelet-lowering agent in essential thrombocythemia. *Curr Hematol Rep*. 2003;2:248-256.
 93. Mazur EM, Rosmarin AG, Sohl PA, et al. Analysis of the mechanism of anagrelide-induced thrombocytopenia in humans. *Blood*. 1992;79:1931-1937.
 94. Fruchtmann SM, Pettit RM, Gilbert HS, et al. Anagrelide: Analysis of long term safety and leukemogenic potential in myeloproliferative diseases (MPDs). *Blood*. 2002;100:70a (abstr.).
 95. 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.
 96. Fruchtmann S, Pettit RM, Gilbert H. Anagrelide Therapy Significantly Reduces Disease Related Symptoms in Patients with Myeloproliferative Disorders. *Blood*. 2003;102:32a (abstr.).

97. Gilbert HS. Historical perspective on the treatment of essential thrombocythemia and polycythemia vera. *Semin Hematol.* 1999;36:19-22.
98. Barosi G, Marchetti M, Azzan C. The Italian registry of myelofibrosis is one year old. *Haematologica.* 2000;85:1121-1122.
99. Chou JM, Li CY, Tefferi A. Bone marrow immunohistochemical studies of angiogenic cytokines and their receptors in myelofibrosis with myeloid metaplasia. *Leuk Res.* 2003;27:499-504.
100. Tefferi A, Mesa RA, Gray LA, et al. Phase 2 trial of imatinib mesylate in myelofibrosis with myeloid metaplasia. *Blood.* 2002;99:3854-3856.
101. 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.
102. 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.
103. Tamanoi F. Inhibitors of Ras farnesyltransferases. *Trends Biochem Sci.* 1993;18:349-353.
104. Vermeulen K, Berneman ZN, Van Bockstaele DR. Cell cycle and apoptosis. *Cell Prolif.* 2003;36:165-175.
105. Cortes J, Albitar M, Thomas D, et al. Efficacy of the farnesyl transferase inhibitor R115777 in chronic myeloid leukemia and other hematologic malignancies. *Blood.* 2003;101:1692-1697.
106. Mesa RA, Tefferi A, Gray LA, et al. In vitro antiproliferative activity of the farnesyltransferase inhibitor R115777 in hematopoietic progenitors from patients with myelofibrosis with myeloid metaplasia. *Leukemia.* 2003;17:849-855.
107. Cervantes F. Prognostic factors and current practice in treatment of myelofibrosis with myeloid metaplasia: an update anno 2000. *Patol Biol (Paris).* 2001;49:148-152.
108. Mesa RA, Hanson CA, Rajkumar SV, et al. Evaluation and clinical correlations of bone marrow angiogenesis in myelofibrosis with myeloid metaplasia. *Blood.* 2000;96:3374-3380.
109. Barosi G, Vittorio R, Margherita M, et al. Spleen neoangiogenesis in patients with myelofibrosis with myeloid metaplasia. *Br J Haematol.* 2004;124:618-625.
110. Elliott MA, Mesa RA, Li CY, et al. Thalidomide treatment in myelofibrosis with myeloid metaplasia. *Br J Haematol.* 2002;117:288-296.
111. Mesa RA, Steensma DP, Pardanani A, et al. A phase 2 trial of combination low-dose thalidomide and prednisone for the treatment of myelofibrosis with myeloid metaplasia. *Blood.* 2003;101:2534-2541.
112. Marchetti M, Barosi G, Balestri F, et al. Low-dose thalidomide ameliorates cytopenias and splenomegaly in myelofibrosis with myeloid metaplasia: a phase II trial. *J Clin Oncol.* 2004;22:424-431.
113. Estey EH, Thall PF. New designs for phase 2 clinical trials. *Blood.* 2003;102:442-448.
114. 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.
115. Guardiola P, Kuentz M, Garban F, et al. Second early allogeneic stem cell transplantations for graft failure in acute leukaemia, chronic myeloid leukaemia and aplastic anaemia. French Society of Bone Marrow Transplantation. *Br J Haematol.* 2000;111:292-302.
116. Deeg HJ, Gooley TA, Flowers ME, et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. *Blood.* 2003;102:3912-3918.
117. Daly AS, McGeer A, Lipton JH. Systemic nocardiosis following allogeneic bone marrow transplantation. *Transpl Infect Dis.* 2003;5:16-20.
118. 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.
119. Guardiola P, Runde V, Bacigalupo A, et al. Retrospective comparison of bone marrow and granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for allogeneic stem cell transplantation using HLA identical sibling donors in myelodysplastic syndromes. *Blood.* 2002;99:4370-4378.
120. Guardiola P, Esperou H, Cazals-Hatem D, et al. Allogeneic bone marrow transplantation for agnogenic myeloid metaplasia. French Society of Bone Marrow Transplantation. *Br J Haematol.* 1997;98:1004-1009.
121. Li Z, Deeg HJ. Pros and cons of splenectomy in patients with myelofibrosis undergoing stem cell transplantation. *Leukemia.* 2001;15:465-467.
122. Helenglass G, Treleaven J, Parikh P, et al. Delayed engraftment associated with splenomegaly in patients undergoing bone marrow transplantation for chronic myeloid leukaemia. *Bone Marrow Transplant.* 1990;5:247-251.
123. Rodrigues CA, Fermino FA, Vasconcelos Y, et al. Refractory chronic GVHD emerging after splenectomy in a marrow transplant recipient with accelerated phase CML. *Bone Marrow Transplant.* 2003;32:333-335.
124. Clouthier SG, Ferrara JL, Teshima T. Graft-versus-host disease in the absence of the spleen after allogeneic bone marrow transplantation. *Transplantation.* 2002;73:1679-1681.
125. 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.
126. Kroger N, Zabelina T, Guardiola P, et al. Allogeneic stem cell transplantation of adult chronic myelomonocytic leukaemia. A report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol.* 2002;118:67-73.
127. de Witte TM, Brand R, van Besien K, et al. Allogeneic Stem Cell Transplantation with Matched Related and Unrelated Donors for Patients with Refractory Anemia: T-Cell Depletion and Reduced Intensity Regimens Are Associated with Increased Relapse Risk. *Blood.* 2003;102:422a (abstr).
128. Barosi G. Myelofibrosis with myeloid metaplasia: diagnostic definition and prognostic classification for clinical studies and treatment guidelines. *J Clin Oncol.* 1999;17:2954-2970.
129. Guardiola P, Anderson JE, Gluckman E. Myelofibrosis with myeloid metaplasia. *N Engl J Med.* 2000;343:659-660.
130. Deeg HJ, Guardiola P. Allogeneic hemopoietic stem cell transplantation in patients with myelodysplastic syndrome or myelofibrosis. *Int J Hematol.* 2002;76 Suppl 2:29-34.
131. 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.
132. Rondelli D, Barosi G, Bacigalupo A, et al. Non-Myeloablative Allogeneic HSCT in High Risk Patients with Myelofibrosis. *Blood.* 2003;102:199a (abstr).
133. 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. *Blood.* 2004;100:70a (abstr).
134. Melear JM, Goldstein RM, Levy MF, et al. Hematologic aspects of liver transplantation for Budd-Chiari syndrome with special reference to myeloproliferative disorders. *Transplantation.* 2002;74:1090-1095.
135. Singh V, Sinha SK, Nain CK, et al. Budd-Chiari syndrome: our experience of 71 patients. *J Gastroenterol Hepatol.* 2000;15:550-554.
136. Valla DC. Hepatic vein thrombosis (Budd-Chiari syndrome). *Semin Liver Dis.* 2002;22:5-14.
137. Lee KH, Lee DY, Won JY, et al. Transcaval transjugular intrahepatic portosystemic shunt: preliminary clinical results. *Korean J Radiol.* 2003;4:35-41.
138. Petersen B. Intravascular ultrasound-guided direct intrahepatic portacaval shunt: description of technique and technical refinements. *J Vasc Interv Radiol.* 2003;14:21-32.
139. Gasparini D, Del Forno M, Sponza M, et al. Transjugular intrahepatic portosystemic shunt by direct transcaval approach in patients with acute and hyperacute Budd-Chiari syndrome. *Eur J Gastroenterol Hepatol.* 2002;14:567-571.
140. Oldhafer KJ, Frerker M, Prokop M, et al. Two-step procedure in Budd-Chiari syndrome with severe intrahepatic vena cava stenosis: vena cava stenting and portocaval shunt. *Am J Gastroenterol.* 1998;93:1165-1166.
141. Perello A, Garcia-Pagan JC, Gilabert R, et al. TIPS is a useful long-term derivative therapy for patients with Budd-Chiari syndrome uncontrolled by medical therapy. *Hepatology.* 2002;35:132-139.
142. Bilbao JI, Quiroga J, Herrero JI, et al. Transjugular intrahepatic portosystemic shunt (TIPS): current status and future possibilities. *Cardiovasc Intervent Radiol.* 2002;25:251-269.
143. Orloff MJ, Orloff MS, Daily PO. Long-term results of treatment of Budd-Chiari syndrome with portal decompression. *Arch Surg.* 1992;127:1182-1187.
144. Pisani-Ceretti A, Intra M, Prestipino F, et al. Surgical and radiologic treatment of primary Budd-Chiari syndrome. *World J Surg.* 1998;22:48-53.
145. Klein AS, Sitzmann JV, Coleman J, et al. Current management of the Budd-Chiari syndrome. *Ann Surg.* 1990;212:144-149.
146. Bismuth H, Sherlock DJ. Portosystemic shunting versus liver transplantation for the Budd-Chiari syndrome. *Ann Surg.* 1991;214:581-589.
147. Emre A, Kalayci G, Ozden I, et al. Mesatrial shunt in Budd-Chiari syndrome. *Am J Surg.* 2000;179:304-308.
148. Orloff MJ, Daily PO, Orloff SL, et al. A 27-year experience with surgical treatment of Budd-Chiari syndrome. *Ann Surg.* 2000;232:340-352.
149. Slakey DP, Klein AS, Venbrux AC, et al. Budd-Chiari syndrome: current management options. *Ann Surg.* 2001;233:522-527.
150. Gisslinger H, Mannhalter C, Pabinger I, et al. High risk of deep vein thrombosis in carriers of the prothrombin gene mutation in patients with polycythemia vera and essential thrombocythemia. *Blood.* 2002;100:796a (abstr).
151. Hasselbalch HC, Bjerrum OW, Jensen BA, et al. Imatinib mesylate in idiopathic and postpolycythemic myelofibrosis. *Am J Hematol.* 2003;74:238-242.
152. Ho AYL, Lim S, Fishlock K, et al. Imatinib mesylate in myelofibrosis: preliminary results show early sustained improvements in platelet counts and splenomegaly. *Blood.* 2002;100:799a (abstr).
153. Odenike O, Hoving K, Sher D, et al. Phase II study of imatinib mesylate (IM) in myelofibrosis with myeloid metaplasia (MMM). *Proc Am Soc Clin Oncol.* 2003;22:585 (abstr).
154. De Angelo DJ, Soiffer RJ, Galinsky I, et al. Imatinib mesylate (Gleevec) for patients with chronic idiopathic myelofibrosis (CIM): a phase II trial. *Blood.* 2003;102:146a (abstr).