Primary myelofibrosis (PMF) is a chronic myeloproliferative disorder associated with an average survival of less than 5 years. Therapy for PMF has used chemotherapeutic agents, immunomodulatory drugs, or biological-response modifiers that have not always been directed at the biological processes that underlie the origins of PMF. Such strategies are palliative and have an uncertain effect on survival. At present, allogeneic stem cell transplantation (ASCT) is the only means of altering the natural history of patients with PMF and provides the only hope for cure of this disorder. Enthusiasm for ASCT in PMF has been muted due to an unacceptable transplantation-related morbidity and mortality in patients receiving fully myeloablative conditioning regimens. Recently, a variety of reduced-intensity conditioning regimens have been utilized in older patients with PMF with significant comorbidities with promising results. Greater understanding of the cellular and molecular events that lead to the development of PMF have provided the opportunity for targeted therapies for PMF. Such therapies must be first evaluated in phase 1/2 trials using a variety of endpoints to assess their efficacy and their potential associated toxicities. The performance of randomized clinical trials comparing these agents to the present standard of care would permit for the first time evidence-based therapeutic decisions to be made for patients with PMF.

Introduction

Primary myelofibrosis (PMF) is a chronic hematologic malignancy characterized by splenomegaly, leukoerythroblastosis, cytopenias, teardrop poikilocytosis, marrow fibrosis, extramedullary hematopoiesis, increased marrow microvessel density, and constitutive mobilization of hematopoietic progenitor cells (HPC) and stem cells (HSC).1,2 PMF has been classified along with essential thrombocythemia (ET) and polycythemia vera (PV) as one of the Philadelphia chromosome–negative (Ph–) myeloproliferative disorders (MPD).1 Unlike PV and ET, which, when appropriately treated, are associated with a prolonged survival, patients with symptomatic forms of PMF have a median survival of less than 5 years.1,2 A syndrome resembling PMF can also develop in patients with PV or ET (post-PV MF or post-ET MF).1,2 Until recently, therapy for PMF has used chemotherapeutic agents, immunomodulatory drugs and biological-response modifiers that are not always directed at the biological processes that underlie the origins or lead to progression of PMF.1,2 The use of such strategies has resulted in palliation of the signs and symptoms of the disease process with an uncertain effect on survival. At present, allogeneic stem cell transplantation (ASCT) is the only means of altering the natural history of patients with PMF and provides the only hope for cure.4 With the greater understanding of the cellular and molecular events that lead to the development of PMF, the possibility for more targeted and effective therapies for this disorder is quickly becoming a reality.

Biology

Fibrosis of the marrow is not unique to PMF and may be secondary to many other disorders.1,2 In idiopathic myelofibrosis (IM), the marrow fibrosis occurs in response to a clonal proliferation of hematopoietic stem cells, which leads to a profound hyperplasia of morphologically abnormal megakaryocytes (MK) and populations of monocytes that release fibrogenic growth factors.1,2 Ultimately, the clinical sequelae of PMF are the consequence of PMF HSC and their progeny and not merely marrow fibrosis. The importance of malignant HSC in PMF is clearly demonstrated by the reversal of the clinical features of this disorder, includ-
ing cytopenias, marrow fibrosis and splenomegaly, which occurs when malignant HSC are replaced by normal HSC following ASCT.4,6

Recently, greater insight into the molecular origins of the myeloproliferative disorders (MPD) has been gained following the discovery of a loss-of-function mutation of an autoinhibitory domain of the Janus kinase family (JAK) of protein tyrosine kinases that is involved in cytokine receptor signaling. The JAK2V617F mutation leads to ongoing phosphorylation of JAK2 activity, which then can bind to a cytokine receptor and promote STAT recruitment.7 This mutation is the likely cause of the hypersensitivity to cytokines that characterizes HPC from each of the MPD. In a mouse marrow transplantation model, marrow cells transduced with JAK2V617F result in a clinical phenotype that closely resembles PV, including erythrocytosis, extramedullary hematopoiesis, and marrow fibrosis.8,9 Although 90% of patients with PV are JAK2V617F positive, approximately 50% of patients with PMF harbor this mutation.8,10 The JAK2V617F mutation is homozygous in 13% of patients with PMF, but in 30% of patients with PV.11 Homozygosity has been attributed to homologous recombination.9 Homozygosity of JAK2V617F in PMF patients is associated with a more frequent occurrence of additional unfavorable cytogenetic abnormalities.12 There are conflicting data as to whether the clinical course of patients with JAK2V617F-positive and JAK2V617F-negative PMF differ.13,14 Additional somatic mutations have been identified in patients with PMF that likely play a role in the biogenesis of PMF. A mutation in the transmembrane domain of the thrombopoietin receptor (cMPL) has been documented in 9% of patients with JAK2V617F-negative PMF (MPLW515L or MPLW515K).15 Pardanani and coworkers have provided data to support the coexistence of MPLS515L, MPLS515K and MPL wild-type (WT) alleles in the same patient.16 Furthermore, 30% of patients with PMF with mutations of cMPL also have the JAK2V617F mutation. By studying archival material, the burden of MPLS515L and MKP515K and JAK2V617 has been shown to remain constant throughout the clinical course of patients with PMF.17 In a murine bone marrow transplantation assay, expression of MPL515L but not WT MPL results in a rapidly progressive, fully penetrable, lethal MPD (18 days) characterized by marked thrombocytosis, leukocytosis, splenomegaly, hepatomegaly, marrow megakaryocytic hyperplasia, and marrow fibrosis, but not erythrocytosis.18 These data suggest that the MPL mutation favors the development of thrombocytosis, while the JAK2V617F mutation favors the development of erythrocytosis. Guglielmelli and coworkers have recently reported that patients with PMF with MPLS515L/K mutations as compared with MPL WT IM were older, present with more severe anemia, and are more likely to require transfusional support.18 Almost 50% of patients with PMF have clonal hematopoiesis but lack mutations of JAK2 or cMPL; these patients, however, have a similar clinical phenotype to patients with the recently identified somatic mutations. It is, therefore, difficult to implicate either of these mutations as the sole cause of PMF.19 It appears, rather, that the genetic origins of PMF represent the culmination of multiple genetic and possibly epigenetic events. Additional genetic events that might play a role in this process are being sought by a number of laboratories.20-22 Comparative genomic hybridization studies have shown that gains of cytogenetic material occur in more than 50% of patients with PMF and most commonly involve gains of 9p, 2q, 3p, chromosome 4, 12q and 13q.22 Furthermore, Dingli et al have identified an unbalanced translocation between chromosomes 1 and 6 with specific breakpoints (t1,6) that they believe to be highly specific to PMF.20 These chromosomal sites may harbor additional genes that play a role in the origins of PMF.

Multiple studies indicate that JAK2V617F and MPL mutational events originate in a cell capable of generating both myeloid and lymphoid cells such as the pluripotent HSC.25-27 The ability of primitive human hematopoietic cells to engraft subletally irradiated immunodeficient mice is the standard surrogate in vivo assay for human HSC. Xu et al have demonstrated that PMF CD34+ cells are capable of engrafting non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice and generating myeloid and B cells that are clonal and JAK2V617F positive and carry patient-specific marker chromosomal abnormalities.28 The differentiation program of PMF CD34+ cells following transplant into NOD/SCID mice was also remarkably different from that of normal CD34+ cells, producing greater numbers of CD34+, CD33+, and CD41+ cells but fewer CD19+ cells.26 This predisposition to produce greater numbers of MK was further explored by incubating PMF and PV CD34+ cells in vitro in the presence of stem cell factor and thrombopoietin (TPO). IM CD34+ cells displayed a far greater proliferative capacity and ability to produce greater numbers of MK that were characterized by a resistance to undergo apoptosis due to overexpression of the anti-apoptotic factor Bcl-XL.27 The MK hyperplasia in PMF, therefore, can be accounted by two characteristics; an increased ability of CD34+ cells to generate MK and the accumulation of MK Bcl-XL.

One of the hallmarks of PMF is profound marrow fibrosis. Collagen type 3, also known as reticulin, and collagen type 1 (COL-1) are the predominant extracellular components of marrow fibrosis in PMF.28 These matrix components are produced by marrow fibroblasts that do not belong to the malignant clone. This deposition of collagen is a result of the release of fibrogenic cytokines by MK and monocytes derived from the malignant stem cell population. TGF-β is a critical cytokine in the development of marrow fibrosis in a variety of clinical disorders, including PMF.28 Furthermore, osteoprotegerin, a secreted inhibitor of bone resorption, has been proposed to play a role in the development of osteosclerosis in PMF by disrupting osteoclastogenesis.30 TGF-β upregulates osteoprotegerin expression and protein synthesis and promotes marrow fibrosis.
by increasing matrix biosynthesis and reducing the rate of matrix degradation. \textsuperscript{31} In an animal model of myelofibrosis due to overproduction of TPO, TGF-β1 knockout mice failed to develop myelofibrosis, while WT mice developed severe fibrosis of the marrow and spleen. \textsuperscript{32} These data conclusively demonstrate the importance of TGF-β in the development of marrow fibrosis. PMF MK have been shown by Ciurea \textit{et al} to produce greater amounts of TGF-β than MK generated \textit{in vitro} from healthy volunteers or patients with PV. \textsuperscript{27} Giraudier and coworkers have previously reported that an immunophilin FK506-binding protein 51 (FKB51) is overexpressed in PMF MK and that FKB51 overexpression leads to activation of NFκB in PMF MKs. \textsuperscript{32} Cells with activated NFκB produce greater amounts of TGF-β. \textsuperscript{33} This effect of NFκB is likely indirect since NFκB cannot directly increase transcription of TGF-β since its promoter does not have NFκB-binding sites. These studies suggest that NFκB or TGF-β inhibitors might be useful in preventing the progression of marrow fibrosis in PMF.

PMF is characterized by the constitutive mobilization of CD34\(^+\) cells as well as endothelial progenitor cells into the peripheral blood. \textsuperscript{1,34-37} Endothelial progenitor cell mobilization predominates during the prefibrotic phase of PMF, while HSC/HPC mobilization occurs characteristically in more clinically advanced phases of the disease. \textsuperscript{34} This dysregulation of HSC trafficking likely ultimately leads to the seeding of extramedullary sites with primitive hematopoietic and endothelial cells, resulting in extramedullary hematopoiesis within the liver and spleen as well as a variety of other organs. Several proteolytic pathways have been documented to play a role in cytokine-mediated stem cell mobilization. \textsuperscript{36,37} Proteins released by activated neutrophils cleave vascular adhesion molecule-1 (VCAM-1) expressed by stromal cells, leading to the disruption of a key adhesive interaction between VCAM-1 and very late antigen-4 (VLA-4) expressed by HSC/HPC. The interaction between stromal cells, endothelial cells, and osteoblast-derived stromal cell derived factor-1 (SDF-1) and the CXC chemokine receptor-4 (CXCR-4) expressed by HSC/HPC is also believed to determine patterns of stem cell trafficking. Proteases, including neutrophil elastase, soluble matrix metalloproteinase-9 (MMP-9) and cell bound MMP-9 have been shown to play a role in the constitutive mobilization of CD34\(^+\) cells that occurs in patients with PMF. \textsuperscript{36} The concentration of soluble VCAM-1, a degradation product of VCAM-1, is elevated in the plasma of patients with PMF and is correlated with the absolute numbers of CD34\(^+\) cells in the peripheral blood of patients with PMF. \textsuperscript{36} Furthermore, CXCR-4 expression by CD34\(^+\) cells is downregulated and plasma SDF-1 levels are elevated, which may account for altered SDF-1/CXCR-4 interactions leading to CD34\(^+\) cell mobilization. \textsuperscript{36} Drugs that target the proteases responsible for constitutive CD34\(^+\) cell mobilization may present an intriguing strategy to prevent the establishment of or to eliminate extramedullary sites of hematopoiesis in patients with PMF.

### Treatment

The treatment of patients with PMF post-PV MF or post-ET PV is largely palliative, frequently using agents that are not directed against the underlying cellular and genetic lesions that lead to this disorder. \textsuperscript{1,2} Since patients with the prefibrotic form of PMF as well as patients with an asymptomatic form of fibrotic PMF enjoy a prolonged survival, therapeutic interventions are usually used only in the patients with symptoms.

The most common symptoms in PMF are the result of severe anemia and/or splenomegaly. \textsuperscript{1,2} Bleeding due to thrombocytopenia, qualitative abnormalities of platelet function, or thrombotic episodes may punctuate the clinical course of patients with PMF. Extramedullary hematopoiesis in the gastrointestinal, central nervous, pulmonary and genitourinary systems may result in symptoms due to enlarging masses or reduction in organ function.

Acute myeloid leukemia (AML) developing in patients with PMF represents a lethal refractory form of leukemia. \textsuperscript{38} In one series of 91 patients, death occurred in 98\% of patients after a median of 2.6 months. \textsuperscript{39} The results with current AML induction therapies in such patients are dismal. Such patients should be referred for ASCT prior to their leukemic transformation. \textsuperscript{38}

Transfusion therapy is the core strategy for the treatment of both anemia and thrombocytopenia. Long-term red-cell transfusion therapy should be accompanied by the initiation of oral iron chelation therapy in order to avoid the long-term consequences of iron overload syndrome. Occasionally, patients have been observed to experience a reduction in the numbers of transfusion following iron chelation therapy. \textsuperscript{39} Although this phenomenon has not been well explained, it might be related to the effects of iron chelation on the production of erythropoietin (EPO) which is determined by HIF-1a levels. Iron-chelating drugs can block the interaction of HIF-1a with von Hippel–Lindau protein. A number of agents, including danazol, erythropoietin, thalidomide, lenalidomide, hydroxyurea, anagrelide, imatinib, 2-chlorodeoxyadenosine, melphalan and busulfan, have been used to correct cytopenias, to halt the progression of splenomegaly or to reduce the size of a site of extramedullary hematopoiesis in patients with PMF. \textsuperscript{1,2,38,41-44} At present, such drug therapy has not been shown to alter the natural history of PMF but is associated with 30\% to 40\% improvement in cytopenias or splenomegaly in the best of situations. In patients with anemia, trials of danazol, erythropoietin, chemotherapy (hydroxyurea or low dose melphalan), or low-dose thalidomide/prednisone or lenalidomide therapy are indicated. \textsuperscript{1,2} The sequence in which these agents are prioritized for use is impossible to determine since there is a glaring lack of randomized trials in this area, which makes evidence-based medical decisions impossible. In fact, in a small randomized double-blind 2B multicenter trial of 52 anemic patients comparing 6 months of therapy with thalidomide 400 mg/day or placebo, there was no difference observed...
after an intent-to-treat analysis between the thalidomide and the placebo groups regarding improvement of hemoglobin levels or reduction of the number of red blood cell transfusions required. Although this study might be criticized because of an excessive dose of thalidomide being used, it highlights two important issues: (1) patients with PMF, for unknown reasons, seem to experience excessive toxicity to doses of drugs frequently tolerated by patients with other hematologic malignancies; and (2) some type of comparison between a candidate agent to the present standard of care is wise before a particular treatment should be widely used. Tefferi and coworkers have recently reported, however, that lenalidomide therapy in PMF associated with del 5(q31) is capable of achieving hematologic remissions accompanied by reduction of numbers of cells bearing marker chromosomes and JAK2V617F. This subset of patients may be uniquely sensitive to this form of immuno-modulatory therapy.

Special mention should be made of the role of splenectomy in treating patients with PMF who have splenic pain, anemia, portal hypertension or thrombocytopenia related to splenomegaly. Following splenectomy, 77%, 50%, 40% and 30% of patients, respectively, have been reported to experience long-term improvement in symptomatic splenomegaly, anemia, portal hypertension, and severe thrombocytopenia. Bleeding and thrombotic complications, however, complicated the course of 25% of patients with PMF, and 6.7% of patients died during the perioperative period. Postoperatively, the major long-term complications include leukocytosis, thrombocytosis, and accelerated hepatic enlargement. Such adverse events can frequently be controlled with the immediate institution of hydroxyurea therapy during the postoperative period or, if unsuccessful, treatment with chlorodeoxyadenosine therapy. The appropriate implementation of splenectomy can result in the improved quality of life of patients with PMF who frequently do not have other therapeutic options. Since splenectomy is associated with significant morbidity and mortality, the physician should only resort to this strategy if thrombocytopenia, anemia or symptomatic splenomegaly are unresponsive to less invasive approaches. Patients should only be considered for this procedure if their performance status allows one to anticipate a favorable surgical outcome with some certainty. Excessive delay of the decision to undergo splenectomy may, however, result in a missed window of opportunity, since the patients may become increasingly debilitated, no longer making them viable surgical candidates.

**Stem Cell Transplantation**

At present, ASCT is the only therapeutic approach with the potential of curing patients with PMF. Selection of an appropriate candidate for transplantation with a poor enough prognosis to justify the risk associated with this approach is a challenging exercise. It should be emphasized that most patients with PMF are too old or have significant co-morbidities that make ASCT an unrealistic alternative. The approach should, however, be considered at present in all patients ≤ 65 years of age or in unusual older patients who are “biologically younger than their stated age.” Clinical and biological parameters that are characteristic of patients with PMF at diagnosis have been used to identify subgroups of patients with different outcomes. The most widely used risk assessment tool to assess the prognosis of patients with PMF is provided in Table 1.

This system (Lille scoring system) was created by Dupriez and coworkers and has proven useful in selecting intermediate- and higher-risk patients as candidates for ASCT. Patients with a Lille score of 0 or patients with a prefibrotic form of PMF should not be considered for transplantation due to their favorable prognosis. Recently, Dingli and coworkers have attempted to improve upon the Lille scoring system by analyzing the characteristics of 160 patients with PMF. They showed that a platelet count of >100 x 10^9/L in patients with IM was associated with an adverse outcome and created a complete blood count scoring system using three parameters: Hgb < 10 gm/dL; WBC < 4 or > 30 x 10^9/L; and platelet count <100 x 10^9/L. Each event was given a score of 1. Patients with a score of 0 had a median survival of 155 months, patients with a score of 1 had a median survival of 69 months, and patients with a score of 2 had a median survival of 23.5 months. Since Dingli and coworkers studied a population of younger patients while Dupriez and coworkers analyzed the outcomes of patients with PMF over the full range of age, it remains impossible to determine which assessment tool is optimal, although both instruments are clearly useful. Most investigators have assumed that the Lille scoring system would also be useful in assessing the prognosis of patients with post-PV or -ET MF. Dingli and coworkers analyzed 66 patients younger than 60 years of age with this form of MF and reported that age less than 45 years and a hemoglobin level less than 10 g/DL were independent risk factors for survival. Unfavorable cytogenetic abnormalities (other than 13q- and 20q-) were shown to be the most useful means of identifying patients with poor outcomes in post-PV or -ET MF.

**Table 1. Risk assessment in primary myelofibrosis.**

| Lille system |
|-----------------|-----------------|-----------------|-----------------|
| Poor prognostic factors |
| Hgb < 10g/dL |
| WBC < 4,000/µL |
| WBC > 30,000/µL |

| Scoring system |
|-----------------|-----------------|-----------------|-----------------|
| No. of factors | Risk group | Percentage of pts. | Median survival (mos.) |
| 0 | Low | 47 | 93 |
| 1 | Intermediate | 45 | 26 |
| 2 | High | 8 | 13 |

Data from Dupriez et al.46
ET MF; patients with such unfavorable cytogenetic abnormalities have a median survival of 12 months as compared with 204+ months for patients with favorable cytogenetics.44 The enthusiasm for allogeneic HSCT in PMF has been muted due to an unacceptable rate of transplantation-related toxicity occurring in patients receiving fully myeloablative conditioning regimens (Table 2). In fact, the transplantation-related mortality (TRM) ranged from 25% to 48% in different studies.46-50 Moreover, advanced patient age at the time of transplantation represented an important prognostic factor; only 14% of PMF transplant patients older than 45 years survived, as compared with 62% of younger patients 5 years after an allogeneic HSCT.50 Since PMF usually affects patients in their fifth to sixth decade, myeloablative allogeneic HSCT, albeit possibly curative, might carry an excessively high risk of death for most patients.

The discovery that allogeneic stem cells can engraft patients prepared with nonmyeloablative doses of radiochemotherapy has led to the rapid development of a variety of reduced-intensity conditioning (RIC) regimens, ranging from partially myeloablative to nonmyeloablative, that have been successfully applied to patients with PMF who would not be candidates for a myeloablative HSCT due to advanced age or co-morbidities.51 (Table 2). A retrospective analysis of RIC transplants in 21 patients with PMF in chronic phase at intermediate/high risk and median age of 53 years showed a TRM, overall survival and event-free survival (EFS) of 10%, 86% and 76%, respectively, after a median follow-up of 2.7 years.3 Similar outcomes were confirmed by other studies.52,53 (Table 2). In particular, Kroger et al performed a pilot prospective study53 in 5 low-risk and 16 intermediate/high-risk patients with PMF (median age, 53 years) who were conditioned with fludarabine, reduced dose busulfan and antithymocyte globulin (ATG), and received an allogeneic HSCT from related (n = 8) or unrelated (n = 13) donors.53 In a recent study, unrelated RIC transplantations were performed in 9 patients with PMF older than 50 years, without use of T-cell–immunomodulating agents such as ATG or alemtuzumab. This strategy resulted in a high rate of severe graft-versus-host disease (GVHD) and 55% overall survival after 2.7 years of follow-up.44 (Table 2).

Overall, the small number of patients in each study does not allow one to select which RIC regimen may be preferable. Nevertheless, alkylating agents, such as melphalan or busulfan, have been successfully used as components of the preparative regimens for allogeneic HSCT in PMF, and the use of a conditioning regimen based on targeted doses of busulfan resulted in a significantly greater patient survival compared with a TBI-based regimen.50 Results of allogeneic RIC HSCT indicate that patients with PMF up to age 65 with advanced disease are candidates for allogeneic HSCT from matched related or unrelated donors. Current data would also indicate that the use of ATG may improve the results in unrelated transplantations. Due to EFS rates greater than 75% in RIC HSCT,4,52,53 it appears clear that elderly patients should be conditioned with RIC regimens; however, it remains controversial whether younger patients with PMF should still receive fully myeloablative regimens. Moreover, patients with a low-risk Lille score who are younger than 65 years of age with a matched related or 10/10 HLA antigen (by high-resolution molecular typing) matched unrelated donor should be followed closely and should undergo an allogeneic HSCT only after progression of disease is observed.

The advantage of splenectomizing a patient prior to allogeneic HSCT for PMF has not been confirmed, although splenectomy has been reported to be associated with more rapid hematologic reconstitution.50,54-55 A retrospective analysis of 26 patients demonstrated no difference in the

<table>
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<tr>
<th>No. pts</th>
<th>Age (y)</th>
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<th>Regimen</th>
<th>TRM at 1 yr (%)</th>
<th>CR (%)</th>
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<td>89</td>
<td>RIC</td>
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</table>

*In 7 of 9 unrelated transplants, antithymocyte globulin (ATG) was not administered; cyclosporine A/mycophenolate mofetil (CsA/MMF) was used as prophylaxis.

Abbreviations: I/H, intensity; TRM, transplantation-related mortality; CR, complete response; OS, overall survival; RIC, reduced-intensity conditioning.

Table 2. Results of allogenic stem cell transplantation in patients with primary myelofibrosis (PMF).
overall and disease-free survivals for splenectomized patients with PMF who received an allogeneic HSCT. At this time, transplantation centers have different policies regarding patients with extensive splenomegaly. The role of splenectomy prior to HSCT should be evaluated in prospective studies. Our initial observations in patients who were prepared with fludarabine and melphalan and then underwent allogeneic HSCT suggest that even severe splenomegaly (> 30 cm longitudinal size by CT scan) can be reduced in a relatively short period of time after transplant (Ciurea S, Hoffman R and Rondelli D, submitted).

**Experimental Therapeutics**

Since patients with intermediate or severe forms of PMF, post-PV MF or post-ET MF have an anticipated short survival and limited therapeutic options beyond allogeneic HSCT, this population should be considered as candidates for a variety of phase 1/2 clinical trials that are currently being pursued. The evaluation of these agents represents the initial step in the development of rational hypothesis-driven therapy for the treatment of patients with PMF. These current strategies (Table 3) will be evaluated using a number of clinical endpoints as well as surrogate biomarkers (Table 4), and the immediate and long-term toxicities associated with their use will be determined. The European Myelofibrosis Network (EUMET) and the International Working Group for Myelofibrosis Research and Treatment (IWG) have independently developed objective criteria by which responses to experimental therapeutic agents might be judged. Since it is likely that a large number of such agents will be evaluated in patients with PMF during the next few years, it is recommended that one of these two means of evaluating a response to a therapeutic agent be implemented so that their relative efficacy can be more easily judged.

**Tyrosine Kinase Inhibitors**

A variety of small-molecule inhibitors of JAK2V617F are under various stages of development. The use of such tyrosine kinase inhibitors are anticipated to be especially effective for the treatment of patients with PV and PMF by reducing the malignant cell burden and therapy, permitting the proliferation of residual normal HSC. Such compounds have also been shown to inhibit cells that harbor the MPL515L-positive cells. Whether such drugs will be effective in treating patients with JAK2V617F- or MPL515L/K-negative IM remains untested. The safety of such compounds will require careful assessment and will likely be determined by the specificity of such candidate compounds. WT JAK2 is required for normal blood cell production. Inhibitors of other JAKs such as Tyk2 and JAK3 are currently being evaluated as immunosuppressive agents, and patients with congenital deficiencies of these other kinases suffer from severe immunodeficiency disorders. Non-specific JAK inhibitors have the potential to lead to cytopenias, bone marrow failure, or immunodeficiency status. Furthermore, 50% of the AML that originate in the context of JAK2V617F-positive MPD have leukemic cells that are JAK2V617F negative, which suggests that these AML may originate from a second malignant clone that is JAK2V617F negative. It is of concern that the use of the JAK2V617F inhibitors might favor the emergence of such leukemias.

**Table 3. Current therapeutic strategies undergoing evaluation for the treatment of primary myelofibrosis.**

- Small-molecule inhibitors of JAK2V617F
- TGF-β inhibitors
- NFκB inhibitors
- Chromatin-modifying agents
- Protease inhibitors
- Bcl-xL inhibitors
- VEGF inhibitors

**Table 4. Therapeutic end points for small molecule therapy of myeloproliferative disorders (MPD).**

- Survival
- Clinical events
- Hematologic parameters
- JAK2 mutational status
- Restoration of polyclonal hematopoiesis
- Bone marrow histology and cytogenetics
- Other biomarkers
by inhibiting osteoprotegerin. Furthermore, bortezomib paired the development of marrow fibrosis by inhibiting TGF-β production, and the development of osteosclerosis by inhibiting osteoprotegerin. Furthermore, bortezomib therapy led to a reduction in the levels of leukocytosis and thrombocytosis in a dose-dependent fashion but did not improve the degree of anemia. Bortezomib therapy also resulted in reduction of the degree of constitutive mobilization of HPC and the degree of splenomegaly, and prolonged survival (89% versus 8% at 52 weeks). Bortezomib therapy is currently being evaluated in a phase 1 trial of patients with PMF.

Chromatin-Modifying Agents
A growing number of investigators have provided data that indicate that epigenetic mechanisms silence genes that impede cellular proliferation or movement in IM. Rossi et al have reported aberrant methylation of a variety of negative regulatory of JAK2 activation/phosphorylation including SHP-1, SOCS-1 and SOCS-3, which have been observed in patients with both JAK2V617F-positive or -negative PMF. Furthermore, Opalinska and coworkers performed high resolution epigenomic mapping of IM cells, which revealed high levels of functionally important methylation. This high rate of methylation in IM suggests that epigenetic silencing of genes may play an important role in the pathogenesis of this disorder. A hypomethylating agent, decitabine, has also been shown to induce expression of TGF-β receptors that have been silenced by hypermethylation and has the potential to restore sensitivity of PMF HSC/HPC to TGF-β.

Shi et al explored the effects of the sequential treatment with the DNA methyl transferase inhibitor decitabine (5azaD) followed by the histone deacetylase inhibitor trichostatin A (TSA) on the behavior of PMF CD34+ cells. Exposure of PMF CD34+ cells to 5azaD/TSA resulted in a reduction of the proportion of JAK2V617F+ HPC in 83% of the patients studied and the reduction in the proportion of homozygous HPCs in 50% of the patients. 5azaD/TSA treatment also led to a dramatic reduction in the number of HPC that contained chromosome abnormalities. Treatment of PMF CD34+ cells with 5azaD/TSA also resulted in the upregulation of CXCR4 expression by CD34+ cells and restoration of their migration in response to SDF-1. These data provide a rationale for sequential therapy with chromatin-modifying agents for patients with PMF. A number of trials using azacitidine or 5azaD alone or in combination with a histone deacetylase inhibitor are either being performed or are in development for patients with PMF.

Anti-VEGF Therapy
PMF is characterized by a profound increase in marrow microvessel density which is associated with increased plasma vascular endothelial growth factor (VEGF) levels. Several groups have explored the possibility that interruption of this increased marrow microvessel density might alter the clinical course of such patients. This hypothesis has served as a foundation for the use of thalidomide and its derivative, lenalidomide, for the treatment of patients with PMF; both agents have potent antiangiogenic and cytokine modulatory activity. Significant response rates have been reported with the use of both agents (~25%). The report of cytogenetic remissions as well as reduction in the JAK2V617F burden in rare patients is especially intriguing. Giles et al have evaluated an oral inhibitor of VEGF receptor tyrosine kinase inhibitor, PTK787/ZK222584, in a phase 1/2 trial of patients with PMF. One patient achieved a complete remission and 5 experienced clinical improvement as judged by International Working Group (IWG) consensus criteria. This oral agent was associated with acceptable toxicity and was judged to be suitable for long-term administration. Giles et al has suggested that this agent might be best used in combination with cytotoxic drugs in the future. Bevacizumab is a humanized monoclonal neutralizing antibody directed against VEGF, which is used to inhibit tumor-related angiogenesis. Bevacizumab is currently approved to treat a variety of metastatic solid tumors but also will be evaluated for therapeutic efficacy in patients with PMF. Bevacizumab binds VEGF and prevents its interaction with its receptors (flt-1 and KDR) present on the surface of endothelial cells.

Inhibitors of Bcl-xL
PMF is characterized by a profound MK hyperplasia. MK from patients with PMF are characterized by upregulation of the antiapoptotic factor Bcl-xL. A number of small molecules with Bcl-xL at subnanomolar concentrations that have recently been identified will be evaluated in several in vitro and in vivo preclinical models to assess their potential for selecting reducing the burden of malignant MK in PMF when used alone or in combination with other chemotherapeutic agents. Furthermore, small interfering RNAs (siRNAs) directed against Bcl-xL have been shown to inhibit cell growth and induce apoptosis of a variety of solid tumors. Such strategies may also be useful for the treatment of PMF.

Inhibitors of Proteases
Neutrophil elastase and MMP-9 have been implicated in altering interactions between PMF CD34+ HSC/HPC with VCAM-1 and SDF-1, respectively, within the narrow microenvironment, leading to constitutive CD34+ cell mobilization and the development of extramedullary hematopoiesis. A variety of MMP-9 inhibitors as well as small-molecule inhibitors of neutrophil elastase are currently being evaluated to treat a variety of clinical disorders, including...
metastasis of solid tumors.36 Such drugs might be valuable in preventing or reducing the extent of extramedullary hematopoiesis when administered alone or in combination with other agents.

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