

Hematopoietic stem cell transplantation and other therapeutic options in primary myelofibrosis: a review and two case reports

Elena V. Morozova, Maria V. Barabanshikova, Tatiana L. Gindina, V.V. Baykov, Boris V. Afanasyev

R.M. Gorbacheva Memorial Research Institute of Children Oncology, Hematology and Transplantation, First St.Petersburg State I. Pavlov University, St.Petersburg, Russia

Dr. Elena V. Morozova, L. Tolstoy St 6/8, 197022, St-Petersburg, Russia,

Phone: +7 (911) 927 8229
 E-mail: dr_morozova@mail.ru

Summary

Primary myelofibrosis (PMF) is BCR/ABL–negative myeloproliferative disorder with splenomegaly, leukoerythroblasts, extramedullary hematopoiesis, reactive bone marrow fibrosis and neoangiogenesis with abnormal cytokine production, generally, affecting elderly persons. PMF may be accomplished by gradual bone marrow failure, splenomegaly, severe general (constitutional) signs, and clinical features of extramedullary hemopoiesis. Acute leukemia develops in up to 30% of PMF. A conventional cytostatic therapy of PMF does not affect the survival rates. Meanwhile, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative procedure for the PMF.

PMF diagnosis is performed by appropriate WHO criteria and includes clinical, morphologic, cytogenetic, and molecular parameters. This disorder should be discerned from other myeloid neoplasias, i.e., polycythemia vera and essential thrombocythemia (ET). Differential diagnosis of PMF should also consider marrow fibrosis caused by other non-neoplastic or neoplastic conditions. In addition to JAK2 V617F or MPL mutations, a more recently found calreticulin (*CALR*) gene marker have been proposed for the MF (and essential patients with MF and ET).

Prognostic aspects are derived from a concept of PMF as a heterogeneous disorder with rather individual manifestation and evolution, with highly variable median survival of up to ≥ 20 years.

An appropriate scoring scale (IPSS) uses five risk factors to predict prognosis and stratify the patients into risk groups. The risk factors for leukemia-free survival include $\geq 3\%$ circulating blasts, platelet count $< 100 \times 10^9/L$, and presence of unfavorable karyotype.

Drug therapy for PMF is not effective, in terms of overall or event-free survival. AlloHSCT for PMF is potentially curative, but still hazardous. Meanwhile, many patients may be observed for sufficient time without any therapy, whereas some of them are effectively managed by conventional treatment.

For low or intermediate-1 risk patients, there is no need for specific therapy in asymptomatic patients. Cytoreductive therapy may be reasonable in cases of extreme leuko- or thrombocytosis. The drug side effects include hepatotoxicity and virilizing effects for androgens, peripheral neuropathy (thalidomide), and myelosuppression (lenalidomide). The first-line therapy with hydroxyurea may reduce the spleen size in 40% of patients, with the spleen response lasting for ca. 1 year. Adverse effects of urea include myelosuppression and mucocutaneous ulcers.

Pegylated interferon is currently applied, early on in the course of ET or PV, with a goal of preventing or delaying fibrosis. The patients with intermediate-2 or high risk disease should be treated with investigational drugs, or alloHSCT. E.g., JAK1/JAK2 inhibitors (Ruxolitinib) are highly effective in PMF or ET MF. At the present time, ruxolitinib is the only FDA-approved JAK2 inhibitor for

MF and the only modern non-HSCT therapy associated with increased survival. AlloHSCT is quite efficient, however, a hazardous intervention, thus being a method of choice for high-risk symptomatic younger patients with PMF. An evident effect of HSCT in MF is a quick restoration of normal trilineage hematopoiesis and functional microenvironment, with rapid and reversal of the myelofibrosis. In conclusion, considering the lack of long-term effective drug therapies for patients with MF, a potential risk of transplant-related complications seems to be proven in patients with DIPSS plus high- or

intermediate 2-risk disease. JAK2 inhibitor provides a unique opportunity of implementing these novel agents into the transplant programs for high-risk patients. The general conclusions are illustrated by two clinical examples from authors' experience showing successful outcomes of allo-HSCT in PMF.

Keywords

primary myelofibrosis, diagnostics, prognosis, treatment, hematopoietic stem cell transplantation

Introduction

Primary myelofibrosis (PMF) is BCR-ABL-negative myeloproliferative disorder characterized by splenomegaly, leukoerythroblastosis, extramedullary hematopoiesis (EMH), circulating CD34 progenitor cells, reactive bone marrow fibrosis, angiogenesis and an abnormal cytokine expression. PMF is a disease usually affecting elderly people. The median age at diagnosis is about 65 years, and fewer than 20% of patients are younger than 50 years [7]. PMF should be distinguished from other closely related myeloid neoplasms including polycythemia vera (PV) and essential thrombocythemia (ET).

The disease course is heterogeneous and can be complicated by progressive bone marrow failure, symptomatic splenomegaly, severe constitutional symptoms, consumption, and clinical manifestations due to extramedullary hemopoiesis [39]. In about 8% to 30% of patients, the disease evolves into acute leukemia [12], [27]. Conventional drug therapy of PMF is merely palliative and does not prolong survival [41]. Allogeneic hemopoietic stem cell transplantation (allo-HSCT) offers the only chance for cure of PMF, but the conventional form of the procedure carries substantial morbidity and mortality and can be offered only to a minority of younger patients. Recently, the introduction of reduced-intensity allo-HSCT has made this therapy available to older patients not eligible for standard allo-HSCT.

Clinical manifestations

Clinical features in PMF are heterogeneous and include severe anemia, marked hepatosplenomegaly, constitutional symptoms (e.g., fatigue, night sweats, fever), cachexia, bone pain, splenic infarct, pruritus, thrombosis, and bleeding. Ineffective erythropoiesis and EMH are the main causes of anemia and organomegaly. Other disease complications include symptomatic portal hypertension that might lead to variceal bleeding or ascites and non-hepatosplenic EMH that might lead to cord compression, ascites, pleural effusion, pulmonary hypertension, or diffuse extremity pain. It is currently assumed that aberrant cytokine production by clonal cells and host immune reaction contributes to PMF-associated

bone marrow stromal changes, ineffective erythropoiesis, EMH, cachexia, and constitutional symptoms [6]. Causes of death include leukemic progression that occurs in approximately 20% of patients but many patients also die of comorbid conditions including cardiovascular events and consequences of cytopenias including infection or bleeding [7].

Laboratory diagnostics

Current diagnosis of PMF is based on WHO criteria and includes clinical, morphologic, cytogenetic, and molecular assessments.

Typical laboratory features in patients with PMF include anemia (28% of PMF cases), peripheral blood leukoerythroblastosis, dacryocytosis, leukocytosis/thrombocytosis, increased lactate dehydrogenase (LDH), excess circulating blasts or CD34 cells, and bone marrow fibrosis, osteosclerosis, and angiogenesis (Figure 1). Differential diagnosis should be performed with polycythemia vera (PV) and essential thrombocythemia (ET). Patients who otherwise fulfill the diagnostic criteria for PV should be labeled as "PV" even if they display substantial bone marrow fibrosis [40].

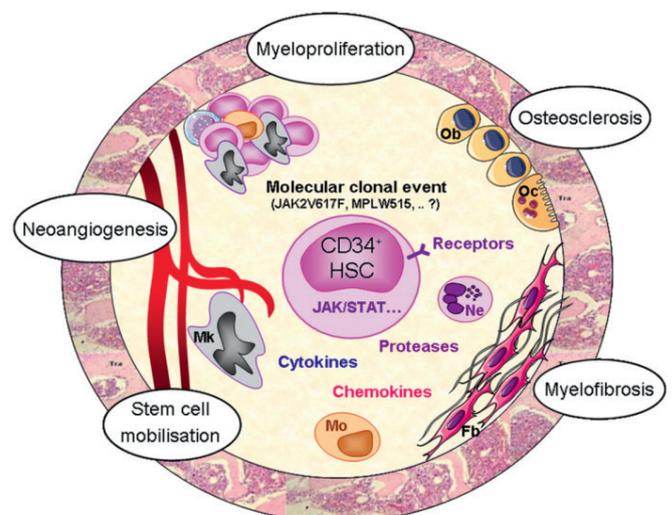


Figure 1. Main aspects of PMF pathogenesis.[24]

Occasionally, overt bone marrow fibrosis might be absent and, in the presence of thrombocytosis, a false diagnosis of ET is made. The possibility of prefibrotic PMF, as opposed to ET, should be considered in the presence of persistently increased serum LDH, anemia, leukoerythroblastosis, increased circulating CD34+ cell count, and marked splenomegaly. It is underscored that the distinction between ET and prefibrotic PMF is clinically relevant because both OS and leukemia-free survival are significantly inferior in the latter [23]. Therefore, prefibrotic myelofibrosis is defined as separate entity in the new version of WHO classification 2016 [3].

The differential diagnosis of PMF should also include bone marrow fibrosis associated with non-neoplastic or other neoplastic conditions, including metastatic cancer, lymphoid neoplasm, or another myeloid malignancy, especially CML, MDS, chronic myelomonocytic leukemia (CMML), or AML. The presence of JAK2 or MPL mutation, with a combined mutational frequency of 70%, reliably excludes reactive bone marrow fibrosis or a nonmyeloid malignancy. More recently, calreticulin (CALR) gene mutations have been reported in patients with MF (and ET) who lack JAK2 V617F or MPL mutations [20]. Nangalia et al. identified a high prevalence of CALR mutations in JAK2/MPL-negative patients with MF. About 56% of patients with JAK2 V617F/MPL-negative MF had CALR mutations [29]. Identification of CALR mutation additionally confirms MF diagnosis.

The absence of BCR-ABL1 excludes the possibility of CML. MDS or CMML should be considered in presence of dyserythropoiesis/dysgranulopoiesis or peripheral blood monocytosis, respectively.

Prognostic factors

PMF is a heterogeneous disease in its presentation and evolution. Median survival is highly variable; a proportion of patients die shortly after diagnosis, whereas a few survive for 2 decades or longer. This fact has stimulated identification of prognostic factors and, as a result, several prognostic systems have been proposed.

The International Prognostic Scoring System (IPSS) uses five risk factors to predict prognosis and assign a patient to a risk group: age older than 65 years; hemoglobin less than 10 g/dL; leukocyte count more than 25×10^9 /L; circulating blood blasts 1% or more; and the presence of constitutional symptoms [7]. The Dynamic IPSS (DIPSS) uses the same five risk factors and has been validated to predict prognosis at any time during the disease course [31].

The DIPSS has been recently modified (DIPSS Plus) with the incorporation of three additional risk factors: red blood cell transfusion needed; platelet count $< 100 \times 10^9$ /L; and unfavorable karyotype [complex or sole or two abnormalities, including + 8, 7/7q, i(17q), inv(3), 5/5q, 12q or 11q23 rearrangement]. The four DIPSS-plus risk categories based on the eight risk factors are low (no risk factors), intermediate-1 (one risk factor), intermediate-2 (two or 3 risk factors), and high (four or more risk factors) with respective median survivals of 15.4, 6.5, 2.9, and 1.3 years [12], table 1. Furthermore, a >80% two-year mortality was predicted by monosomal karyotype, inv(3)/i(17q) abnormalities, or any two of circulating blasts >9%, leukocytes 40×10^9 /L or more, or other unfavorable karyotype. Patients with the latter characteristics are operationally assigned a “very high risk” category and might be better served by immediate consideration for alloHSCT [42].

Several molecular prognostic markers might be soon included in DIPSSplus. A. Tefferi et al. reported DIPSS-plus independent prognostic significance for calreticulin (CALR) (favorable) and ASXL1 (unfavorable) mutations. Survival was the longest in CALR+ASXL- (median 10.4 years) and the shortest in CALR-ASXL1+ patients (median, 2.3 years; HR, 5.9; 95%CI, 3.5–10.0). The CALR/ASXL1 mutations-based prognostic model was DIPSS-plus independent ($P < 0.0001$) and effective in identifying low-/intermediate-1-risk patients with shorter (median, 4 years) or longer (median 20 years) survival and high-/intermediate-2-risk patients with shorter (median, 2.3 years) survival. Multivariable analysis distinguished CALR-ASXL1+ mutational status as the most significant risk factor for survival: HR 3.7 vs 2.8 for age >65 years vs 2.7 for unfavorable karyotype [45]. In a large multicenter study involving 879 patients with PMF other molecular markers (SRSF2, EZH2, TET2, DNMT3A, CBL, IDH1, IDH2, MPL and JAK2) showed no prognostic significance [45].

Survival in PMF was also affected by increased serum IL-8 and IL-2R levels as well as serum-free light chain levels, both independent of DIPSS-plus [30], [43].

Risk factors for leukemia-free survival include $\geq 3\%$ circulating blasts, platelet count $< 100 \times 10^9$ /L, and presence of unfavorable karyotype. Although DIPSS has been shown to predict leukemia-free survival in the aforementioned DIPSS-plus study of 793 patients with PMF, the only two risk factors for leukemic transformation were unfavorable karyotype and platelet count $< 100 \times 10^9$ /L; 10-year risk of leukemic transformation were 12% in the absence of these two risk factors and 31% in the presence of one or both risk factors [32].

Table 1. Prognostic risk models in PMF [7, 10, 12, 31]

Lille				
Hemoglobin <100 g/l	WBC<4×10 ⁹ /l WBC>30×10 ⁹ /l	Circulating blast 1% or more	Age above 65 years	
IPSS				
Hemoglobin <100 g/l	WBC>25×10 ⁹ /l	Circulating blast 1% or more	Age above 65 years	Constitutional symptoms
DIPSS				
Hemoglobin <100 g/l	WBC>25×10 ⁹ /l	Circulating blast 1% or more	Age above 65 years	Constitutional symptoms
DIPSSplus				
Hemoglobin <100 g/l	WBC>25×10 ⁹ /l	Circulating blast 1% or more	Age above 65 years	Constitutional symptoms* +thrombocytopenia <10×10 ⁹ /l +RBC transfusion need +unfavorable karyotype**

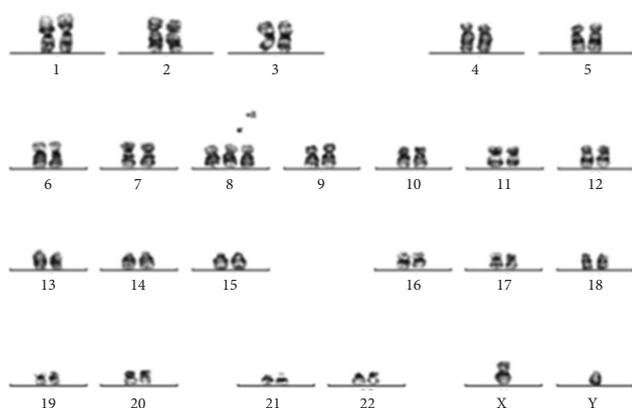


Figure 2. Chromosomal abnormalities in PMF. Typical chromosomal abnormality in patient with PMF 47XY, XY,+8[8]/46,XY[12].

Treatment strategy

Current drug therapy for PMF is not curative and has not been shown to prolong survival. AlloHSCT for PMF is potentially curative but dangerous; transplant-related death or severe morbidity occurs in a high percent of transplanted patients. As a result, more and more patients with PMF (or post-PV/ET MF) are seeking treatment with novel drugs. However, it should be noted that many patients can be ob-

served without any therapeutic intervention and some can be effectively managed by conventional drug therapy.

Management of low or intermediate-1 risk patients

There is no evidence to support the specific therapy in asymptomatic patients with low or intermediate-1 risk disease [41]. Some patients with low or intermediate-1 risk might require therapy for symptomatic anemia, splenomegaly, non-hepatosplenic EMH, bone pain, EMH-associated pulmonary hypertension, or constitutional symptoms (e.g., fatigue, night sweats, and pruritus). In addition, cytoreductive therapy is reasonable but not mandated in the presence of extreme leukocytosis or thrombocytosis.

MF-associated anemia is usually treated with androgens, prednisone (0.5 mg/kg/day), danazol (600 mg/day) [6], thalidomide, or lenalidomide (10mg/day) [33].

Drug side effects include hepatotoxicity and virilizing effects for androgens, peripheral neuropathy for thalidomide, and myelosuppression for lenalidomide.

Response rates to each one of the aforementioned drugs are in the vicinity of 15-25% and response durations average about one to two years. Lenalidomide works best in the presence of del(5q31) [38].

* Constitutional symptoms constitute weight loss > 10% of baseline value in the year preceding diagnosis, unexplained fever, or excessive sweats persisting for > 1 month.38

**Unfavorable karyotype constitutes complex karyotype or sole or 2 abnormalities that include 8, 7/7q, i(17q), inv(3), 5/5q12p, or 11q23 rearrangement.

First-line therapy for MF-associated splenomegaly is hydroxyurea, which is effective in reducing spleen size by half in approximately 40% of patients [26]. Spleen response to hydroxyurea lasts for an average of one year and treatment side effects include myelosuppression and mucocutaneous ulcers.

Interferon- α could be used in symptomatic patients with PMF. Interferon suppresses hematopoietic progenitors, bone marrow fibroblast progenitors and platelet-derived growth factor. The advent of a pegylated version of interferon has renewed interest in this class, perhaps for using early on in the course of ET or PV, with a goal of preventing or delaying fibrosis [19]. Long-term follow-up of 62 French and Belgian patients with MF treated with interferon- α has been recently reported; 46% experienced an improvement in splenomegaly, 82% experienced a mitigation in MF symptoms, and 73%, 64% and 78% of patients experienced an improvement in anemia, leukocytosis and thrombocytosis, respectively [16]. Complementing this study was a prospective trial of 32 lower-risk patients with early MF treated with recombinant or pegylated interferon [36]. An overall response rate of 78% was observed, with 3 complete remissions (9.4%), 12 partial remissions (37.5%), 3 clinical improvements (9.4%) and 7 with (22%) stable disease. Follow-up bone marrow biopsy results were available for 22 patients. Twelve patients had a reduction in cellularity that occurred after a median treatment duration of 2 years. Three patients, all of whom also experienced reductions in splenomegaly, had significant improvements in megakaryocyte morphology, marrow architecture and reductions of reticulin and collagen fibrosis (grade 3 to 1).

Management of intermediate-2 or high risk disease

PMF patients with high or intermediate-2 risk disease should be considered for investigational drug therapy or alloHSCT.

JAK1/JAK2 inhibitors are highly effective in patients with MF. Ruxolitinib was evaluated in 153 patients with PMF or post-PV/ET MF, in a Phase-1/2 study [47]. Dose limiting toxicity (DLT) was thrombocytopenia and the maximum tolerated dose was either 25 mg twice-daily or 100 mg once-daily. Adverse events included thrombocytopenia, anemia, and a “cytokine rebound reaction” upon drug discontinuation, characterized by acute relapse of symptoms and splenomegaly [47]. Non-hematologic adverse events were infrequent. Grade 3/4 thrombocytopenia or anemia (in transfusion-independent patients at baseline) respectively occurred in 39% and 43% of patients receiving the drug at 25 or 10 mg twice daily.

Among all evaluable patients, 44% experienced 50% decrease in palpable spleen size. Improvement in constitutional symptoms (fatigue, pruritus, abdominal discomfort, early satiety, night sweats, and exercise tolerance) and weight gain were seen in the majority of patients. Four (14%) of 28 transfusion-dependent patients became transfusion-independent. The drug's effect on JAK2V617F allele burden or bone

marrow pathology was negligible but a major reduction in proinflammatory cytokines (e.g., IL-1RA, IL-6, TNF- α , MIP-1b) was documented and coincided with improvement in constitutional symptoms. Two randomized studies comparing ruxolitinib with either placebo or best supportive care have now been published [11], [47]. In the COMFORT-1 trial that compared the drug with placebo (n5309), the spleen response rate was approximately 42% for ruxolitinib versus <1% for placebo.

In addition, about 46% of patients experienced substantial improvement in their constitutional symptoms. However, the benefit of the drug was antagonized by ruxolitinib-associated anemia (31% vs. 13.9%) and thrombocytopenia (34.2% vs. 9.3%). In the COMFORT-2 trial that compared the drug with “best available therapy” (n5219), the spleen response was 28.5% with ruxolitinib vs. 0% otherwise but the drug was detrimental in terms of thrombocytopenia (44.5% vs. 9.6%), anemia (40.4% vs. 12.3%), and diarrhea (24.0% vs. 11.0%). The long-term outcome of ruxolitinib therapy in MF was recently reported and disclosed a very high treatment discontinuation rate (92% after a median time of 9.2 months) and the occurrence of severe withdrawal symptoms during ruxolitinib treatment discontinuation (“ruxolitinib withdrawal syndrome”) characterized by acute relapse of disease symptoms, accelerated splenomegaly, worsening of cytopenias, and occasional hemodynamic decompensation, including a septic shock-like syndrome [47].

Presently, ruxolitinib is the only FDA-approved JAK1/2 inhibitor for MF and the only non-HCT therapy to date associated with a proven survival benefit. In the phase III COMFORT-I study ruxolitinib was associated with an overall survival benefit relative to placebo in patients with intermediate-2 or high-risk MF. With median follow-ups of 149.1 and 149.3 weeks for the ruxolitinib and placebo arms, respectively, the hazard ratio for overall survival continued to favor patients originally randomized to ruxolitinib compared with those originally randomized to placebo [hazard ratio 0.69 (95% CI: 0.46–1.03); $P=0.067$]. Different long-term outcomes have been reported by the Mayo Clinic and the MD Anderson Cancer Center (MDACC) in follow-up of their own institutional cohorts enrolled in the phase 1/2 study of ruxolitinib. Including adjustment for the Dynamic International Prognostic Scoring System score, the Mayo Clinic reported no significant difference in the survival rate of their 51 ruxolitinib-treated patients compared with a cohort of 410 patients with PMF who were treated with standard therapy at their center in the most recent 10-year period [46].

MDACC undertook a similar analysis, comparing the long-term outcomes of their patients with a historical control cohort of 310 patients culled from 3 databases who would have met eligibility for the study [48]. OS was significantly improved in ruxolitinib-treated patients compared with historical controls adjusted for International Prognostic Scoring System risk group. The 1-, 2-, and 3-year survival rates in high-risk patients treated with ruxolitinib were superior to the historical control group and a nonsignificant trend in survival was observed between intermediate-2-risk ruxolitinib treated and historical control patients.

In the absence of hematologic remissions, significant reduction in BM fibrosis and/or V617F allele burden, nor any proven modification of leukemia-free survival, other factors may explain the emerging survival advantage with ruxolitinib. Foremost is the enhancement of performance status related to reduction of splenomegaly and improvement of constitutional symptoms.

These data suggest that the survival advantage associated with ruxolitinib may be partly explained by reversion of the catabolic state associated with MF.

Allogeneic stem cell transplantation

AlloHSCT is arguably one of the riskiest interventions in modern medicine, so careful patient selection is of paramount importance. Transplantation for PV and ET, overall associated with a normal or near-normal life expectancy, is not indicated. On the other hand, alloHSCT should be an initial consideration for all patients with MF when first evaluated, and is the treatment of choice for high-risk symptomatic younger patients. What is fascinating and tantalizing in such cases is the capacity for this approach to restore normal trilineage hematopoiesis in a grossly perturbed marrow microenvironment, with rapid and striking reversal of the fibrosis that is the hallmark of this neoplasm [22].

Although a broad range of conditioning regimens of different intensities has been developed, the initial studies of alloHSCT for MF used high-intensity myeloablative conditioning (MAC) regimens, usually including total body irradiation (TBI) or busulfan. For MAC regimens, graft failure rates of less than 5% to 30% have been reported, reflecting the heterogeneity in patient populations and specific conditioning regimens [14], [18]. Published TRM rates of 10% to 35% at 1 year and OS from 30% to 67% at 5 years have been reported [5, 9, 13, 21, 25, 28].

The largest study to date was reported from the Center for International Blood and Marrow Transplant Research (CIBMTR), analyzing data in 289 patients with PMF [5]. Patients underwent a transplant between 1989 and 2002 at 118 centers, with a variety of conditioning regimens. A total of 162 patients received an HLA-matched sibling transplant, 101 received HLA-matched unrelated donor (URD) transplants, and 26 received transplants from HLA nonidentical related donors. Most of the patients received bone marrow as the stem cell source, and 83% were conditioned with a MAC regimen. The 100-day TRM was 18% for HLA-matched sibling patients and 33% for the URD patients. The graft failure rate was 9% for HLA-matched sibling transplants and 20% for URD transplants. Splenomegaly did not impact the graft failure rate. Graft-versus-host-disease (GVHD) grades II to IV occurred in 43% of sibling patients and 40% of the URD patients. The OS at 5 years was 37% for sibling transplants and 30% for URD transplants. Relapse-free survival (RFS) at 5 years was 33% for recipients of an HLA-identical sibling allografts and 27% for recipients of URD transplants. Positive predictors for survival included HLA-identical sibling

donors, performance status greater than 90%, and absence of peripheral blood blasts at the time of transplantation. Patients who had a poor Karnofsky score, peripheral blood blasts, or received a transplant from a URD had a 15% probability of 3-year survival.

The largest prospective multicenter study to evaluate transplantation for MF was conducted through the European Group for Blood and Marrow Transplantation (EBMT) using a reduced intensity strategy [21]. Using the combination of fludarabine, busulfan (10 mg/kg) and antithymocyte globulin with a standard prophylactic immunosuppressive regimen, 98% of patients engrafted, with a nonrelapse mortality of 16% at 1 year. In addition, the estimated 5-year overall survival was 67%. Older age (over 55) and a mismatched donor adversely influenced survival. Subsequent post hoc analyses showed that JAK2 V617F negative disease also carried adverse prognostic significance [2]. These generally favorable results were mirrored histologically in those patients who had serial bone marrow biopsies following transplantation. These studies showed near or complete resolution of fibrosis in 69% and 93% of patients by day 100 and day 365. An additional notable finding from the multivariable analysis was that a history of splenectomy was associated with a higher risk of relapse.

JAK1/JAK2 inhibition as pre- and posttransplant therapy

Most of MF patients are in active disease phase at the time of allo-HSCT and have severe splenomegaly and constitutional symptoms. High disease burden may be one of the reasons why the results of allo-HSCT in MF patients are not satisfactory in some cases. Furthermore, a lot of studies report relatively high incidence of graft failure and poor graft function in MF patients compared to some other hematologic disorders [1, 5, 21, 34]. Several studies reported higher median time to neutrophil and platelets engraftment in patients with splenomegaly compared to patients with splenectomy, but this difference showed no significant impact on overall survival [1, 21]. Other data suggest that splenomegaly is an independent predictor of inferior overall survival after allo-HSCT [4]. Alchalby et al. showed that the other feature of active disease phase – constitutional symptoms also decrease OS after allo-HSCT [2].

Using JAK1/2 inhibitors to reduce spleen size and constitutional symptoms before HCT may be useful in improving transplant outcome. In a study by a German group, 14 patients received allo-HCT following a median of 6.5 months treatment with ruxolitinib [17]. Under ruxolitinib therapy, spleen size was reduced in 64% of patients and engraftment was achieved in 93% of patients. TRM was 7% and survival 79%, but the median follow-up was only 9 months. Another German group reported a retrospective analysis of results in 22 patients with PMF or after ET/PV MF who had received a median of 97 days of ruxolitinib before alloHSCT [37]. At the time of transplant, 86% had improvement in constitutional symptoms and 41% had a major response in spleen size. With a median follow-up of 12 months, the 1-year OS was 81% and PFS was 76%.

Recently Shanavas et al. has reported results of large multi-center retrospective study, which tested efficacy of pretransplant JAK1/2 inhibitors therapy [35]. 100 patients with MF who undergo alloHSCT after JAK1/2 inhibitors therapy were included in this study. Multivariate analysis showed that response to JAK1/2 inhibitors therapy significantly improves OS (p=0,03). Thus 2-years OS was 91% in patients who achieved clinical improvement and 32% in those patients who progressed to leukemic transformation on JAK1/2 inhibitors therapy.

The experience in administrating JAK1/2 inhibitors after allo-HSCT in MF patients is very scarce. We found only one report about posttransplant ruxolitinib therapy which was administrated to prevent relapse in 3 MF patients and 1 CMML patient [44]. Authors report no adverse events of toxicity during posttransplant therapy.

The beneficial impact of JAK1/2 inhibitors on transplant outcomes may be explained by its ability to modulate disease status compared to the other conventional therapies [47]. Pretransplant ruxolitinib therapy may reduce splenomegaly

and constitutional symptoms and possibly reduce the graft failure rate. At the same time ruxolitinib reduce the proinflammatory and proangiogenic cytokines overproduction in MF patients [46]. Through this effect it can modulate immune response and reduce dendritic cells activation [15], neutrophils activation [49] and migration of alloreactive T-cells [8]. Possibly ruxolitinib pre- and posttransplant therapy may reduce GVHD rate and improve transplant results in MF-patients (Figure 3). Currently ruxolitinib is effectively used for treatment of steroid-refractory acute and chronic GVHD [49]. Choi et al. showed that pharmacologic blockade of JAK1/2 results in reduction of CXCR3 expression in T cells, mitigation of GvHD after allo-HSCT and preservation of graft versus leukemia effect in mouse models [8]. The authors suggest that JAK1/2 inhibitors might be a promising therapeutic approach to achieve the beneficial anti-leukemia effect and reduce GVHD rate in allo-HSCT.

Whether JAK1/2 inhibitors treatment before HCT can consistently improve patient performance status, the degree of splenomegaly, GVHD rate and lead to more favorable outcomes is an important area of ongoing investigation.

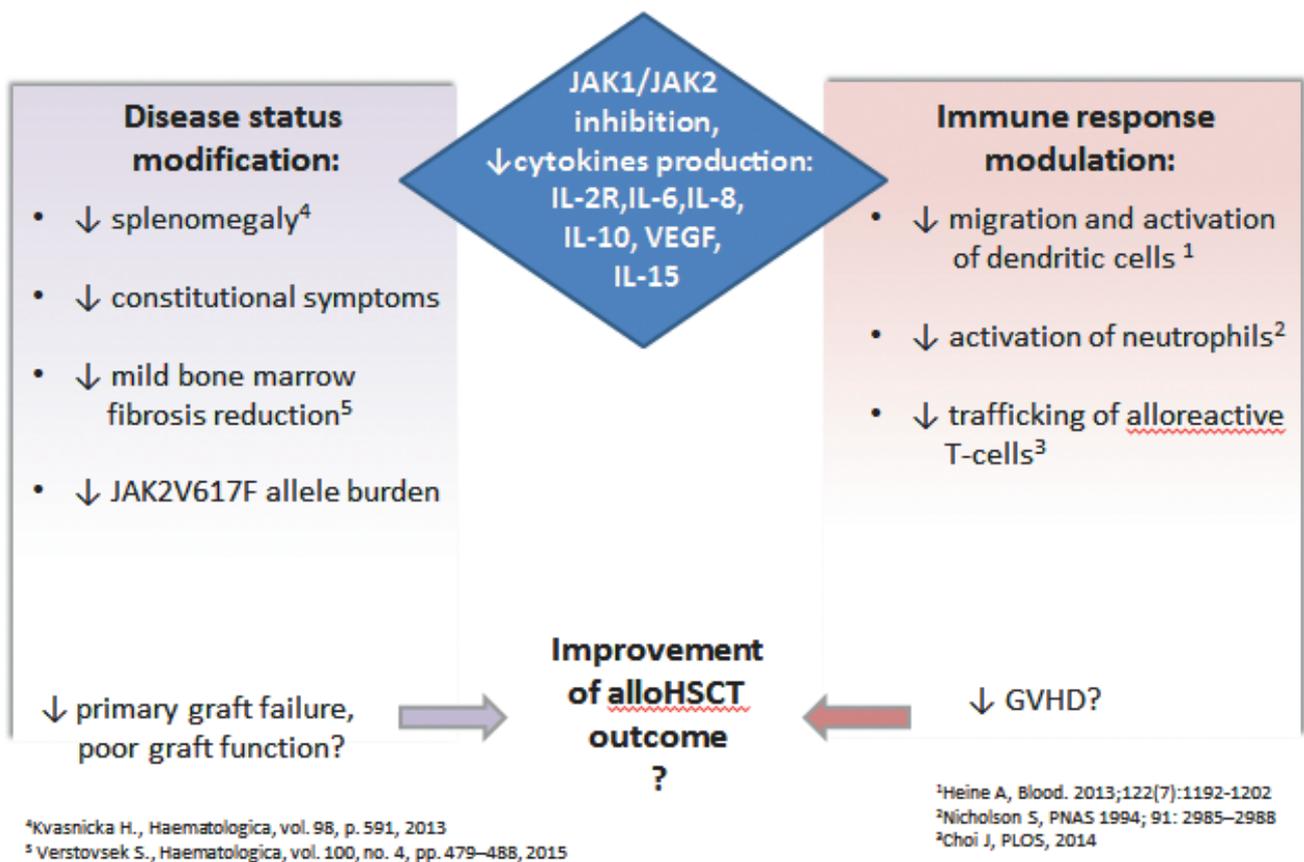


Figure 3. Pre-and posttransplant JAK1/2 inhibitors therapy.

Clinical case descriptions

Here we report two female patients with postpolycythaemic myelofibrosis treated with allogeneic stem cell transplantation.

Patient No.1 was a 46-year old woman with polycythaemia vera diagnosed 5 years prior to transplantation in 2010. At presentation she suffered from splenomegaly-associated symptoms – occasionally episodes of pain in the left upper quadrant. Complete blood count showed hemoglobin

189 g/L; WBC count, $21.5 \times 10^9/L$; myelocytes, 7%; metamyelocytes; 2%; band forms; 11%; segmented forms, 61%; monocytes, 10%; lymphocytes, 9%; platelets, $268 \times 10^9/L$. Ultrasound scan detected enlarged spleen (193x82 mm).

Bone marrow molecular biology analysis revealed a JAK2V617F mutation. Bone marrow cytogenetic analysis has shown a clonal aberration (47,XX+8) observed in 20/20 mitotic cells.

Erythrocytapheresis and hydroxyurea therapy were subsequently performed a symptomatic treatment, due to high hemoglobin level and splenomegaly.

After 5 years of follow-up, the patient developed the disease progression to myelofibrosis as documented by peripheral blood blastosis, LDH elevation, and histologically verified bone marrow fibrosis (Grade 2/3). At the time of progression, the JAK2 V617F-allele burden was 55% of the wild-type level. Thus, the patient was classified into the intermediate-2 risk category corresponding to DIPSSplus and, therefore, allo-HSCT was indicated.

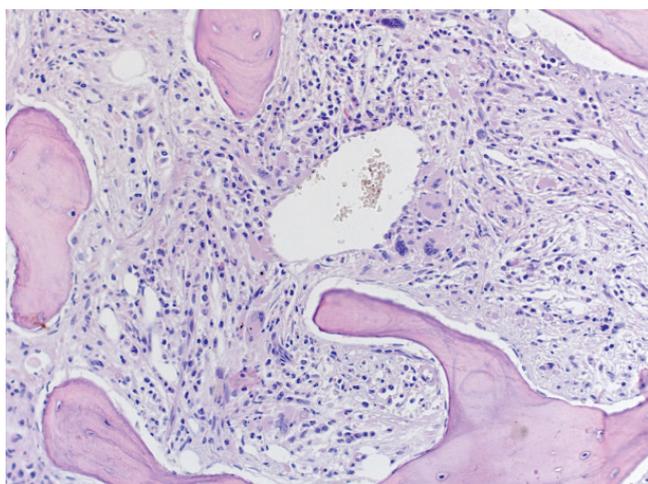
To reduce splenomegaly, were performing pre-transplant ruxolitinib therapy for 3 months. The patient achieved clinical improvement, as evidenced by significant spleen reduction and disappearance of blasts in periphery. At that clinical stage, we performed allogeneic stem cell transplantation from 9/10 -HLA matched unrelated donor in December 2015. We used G-SCF-mobilized peripheral stem cells as stem cell source and transfused 6.6×10^9 CD34-positive cells per kg weight. Conditioning regimen consisted of fludarabine (180 mg/m²), busulfan (10 mg/kg p.o.). The patient continued to take ruxolitinib during conditioning until day -1. Post-transplant cyclophosphamide was administered at 100 mg/kg at day +3, +4, and ruxolitinib 10 mg was used from day +5 as GVHD prophylaxis. During early post-transplant period, the patient developed non-severe veno-occlusive liver disease. This complication resolved later spontaneously. Neutrophil and platelet engraftment, like as independence

on red blood cell transfusion was achieved at day 20-21 after transplant. Complete hematological, cytogenetic and molecular remission was documented at day +30. A near-complete resolution of the bone marrow fibrosis was achieved at day +100. By the day +80, with continued ruxolitinib prophylaxis, the patient developed an overlap GVHD with moderate liver and skin involvement. The GVHD was resolved following Cyclosporine A administration. At 6 months post-transplant, she is alive and in complete remission.

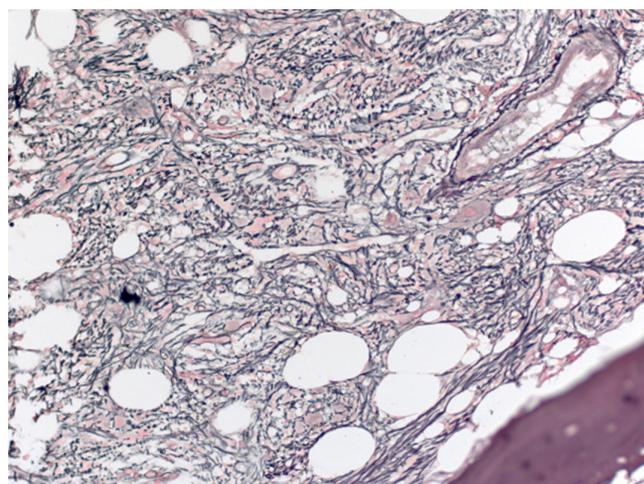
Patient No.2 who received pre-transplant ruxolitinib therapy at our Institute was a 57-year old woman with primary myelofibrosis. At presentation in 2010 she had splenomegaly-associated symptoms, fatigue. CBC showed Hb at 111 g/L; platelets, $256 \times 10^9/L$; WBC, $6.4 \times 10^9/L$; myelocytes, 2%; metamyelocytes, 2%; band forms, 9%; segmented forms, 52%; monocytes, 7%; lymphocytes, 27%; eosinophils, 1%. She also had marked splenomegaly (20x10 mm).

In bone marrow karyotype, a clonal chromosome aberration, 46,XX t(11;16), was found. Molecular biology analysis of bone marrow cells revealed a typical CALR- mutation. Bone marrow trephine biopsy showed typical signs of overt primary myelofibrosis, i.e., atypical megakaryocytes with cluster formation and paratrabecular localization, along with Grade 3 bone marrow fibrosis (Fig. 4). The patient was attributed to the low-risk IPSS category, and started to receive Interferon- α and hydroxyurea for symptomatic splenomegaly. Despite therapy, she progressed 4 years later, as confirmed by peripheral blood blastosis and anemia.

Thus, the patient exhibited new unfavorable risk factors, having been reclassified to intermediate-2 risk group, according to DIPSS and DIPSSplus. Therefore, allogeneic stem cell transplant from full-matched unrelated donor was decided. Pre-transplant ruxolitinib therapy being performed for 6 months, in order to reduce splenomegaly. Splenomegaly was still prominent after 3 months of treatment, and pulse therapy with Dexametasone was also performed, resulting into the disease stabilization.



A



B

Figure 4. Bone marrow trephine biopsy before alloHSCT.

A: Megakaryocyte proliferation with cluster formation. H&E staining.

B: Bone marrow fibrosis grade 3. Gomori staining.

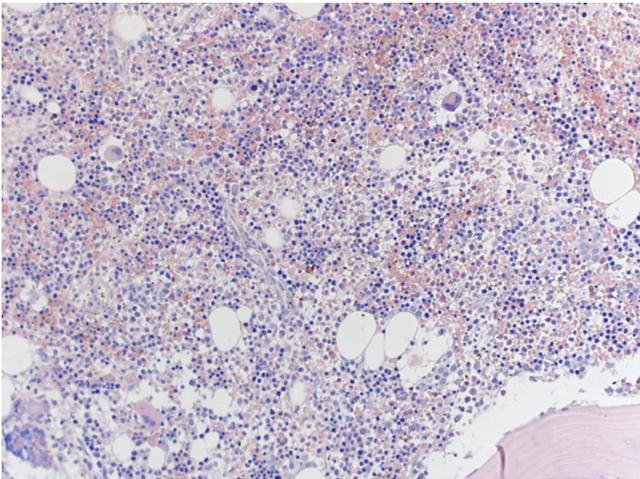


Figure 5. Bone marrow trephine biopsy day +110 after alloHSCT.

Normalization of bone marrow architecture, cellularity and megakaryocyte morphology. H&E staining.

AlloHSCT was performed in March 2015. We used G-SCF-mobilized peripheral stem cells as a stem cell source and transfused 5.1×10^9 CD34-positive cells per kg weight. Conditioning regimen consisted of Fludarabine (180 mg/m²) busulfan (10 mg/kg p.o.). Ruxolitinib was stopped at the first day of conditioning regimen. Post-transplant cyclophosphamide 100 mg/kg at day +3, +4, tacrolimus and mycophenolate mofetil from day +5 were used as GVHD prophylaxis. Neutrophil and platelet engraftment was achieved at day 42 after transplant. Red blood cell transfusion independency was established at day 141 post-transplant. Complete hematological, cytogenetic and molecular remission was revealed at day +27. A near-complete resolution of bone marrow fibrosis was achieved at day +110 (Fig.5). There were no signs of acute or chronic GVHD. By 14 months post-transplant, the patient is alive and remains in complete remission.

Conclusion

Primary myelofibrosis is a myeloproliferative neoplasm characterized by progressive clinical course. Several prognostic models and prognostic factors may help to differentiate this heterogeneous entity into different risk categories and predict survival in PMF patients. Prognostic models should be widely used to make PMF therapy more personalized. New target therapy with JAK1/JAK2 inhibitors showed high efficacy in reducing constitutional symptoms and splenomegaly in significant part of patients. Nevertheless alloHSCT remains to be the only treatment option with curative potential. Janus kinase inhibitors may be successfully used as “bridging” therapy before transplantation and possibly has beneficial impact on transplantation outcomes. Further trials are needed to validate Janus kinase inhibitors efficacy in pre- and posttransplant settings.

Considering the lack of long-term effective drug therapies for patients with MF, the potential risk of transplant-related complications seems justified in patients with DIPSS plus high- or intermediate 2–risk disease. The JAK2 inhibitor era

provides a unique opportunity to begin to incorporate novel agents into the transplant algorithm for high-risk patients. Clinical trials, which examine the best way to use these agents in concert with allo-HCT and the optimal timing, will be the key to providing the best therapy for patients.

Conflict of interest

The authors declare no conflict of interest.

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