Biol Blood Marrow Transplant 20 (2014) 812–815

Dynamic of Bone Marrow Fibrosis Regression Predicts Survival after Allogeneic Stem Cell Transplantation for Myelofibrosis

Nicolaus Kröger [1,*], Tatjana Zabelina [1], Haefaa Alchalby [1], Thomas Stübig [1], Christine Wolschke [1], Francis Ayuk [1], Natascha von Hünerbein [1], Hans-Michael Kvasnicka [2], Jürgen Thiele [3], Hans-Heinrich Kreipe [4], Guntram Büsche [3]

[1] *Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
[2] *Institute of Pathology, University Hospital of Frankfurt, Frankfurt, Germany*
[3] *Institute of Pathology, University of Cologne, Cologne, Germany*
[4] *Institute of Pathology, Hannover Medical School, Hannover, Germany*

Article history:
Received 16 January 2014
Accepted 21 February 2014

Key Words:
Bone marrow fibrosis
Fibrosis regression
Myelofibrosis
Allogeneic stem cell transplantation
JAK2 mutation

A B S T R A C T

We correlate regression of bone marrow fibrosis (BMF) on day 30 and 100 after dose- reduced allogeneic stem cell transplantation (allo-SCT) in 57 patients with primary or post–essential thrombocythemia/polycythemia vera myelofibrosis with graft function and survival. The distribution of International Prognostic Scoring System (IPSS) risk score categories was 1 patient with low risk, 5 patients with intermediate-1 risk, 18 patients with intermediate-2 risk, and 33 patients with high risk. Before allo-SCT, 41 patients (72%) were classified as XXX [myclofibrosis (MF)]-3 and 16 (28%) were classified as MF-2 according to the World Health Organization criteria. At postengraftment day +30 (±10 days), 21% of the patients had near-complete or complete regression of BMF (MF-0/-1), and on day +100 (±20 days), 54% were MF-0/-1. The 5-year overall survival rate at day +100 was 96% in patients with MF-0/-1 and 57% for those with MF-2/-3 ($P = .04$). There was no difference in BMF regression at day +100 between IPSS high-risk and low/intermediate-risk patients. Complete donor cell chimerism at day +100 was seen in 81% of patients with MF-0/-1 and in 31% of those with MF-2/-3. Patients with MF-2/-3 at day +100 were more likely to be transfusion-dependent for either RBCs ($P = .014$) or platelets ($P = .018$). Rapid BMF regression after reduced-intensity conditioning allo-SCT resulted in a favorable survival independent of IPSS risk score at transplantation.

INTRODUCTION

Bone marrow fibrosis (BMF) is a hallmark of primary or post–essential thrombocythemia (ET)/polycythemia vera (PV) myelofibrosis [1]. The fibrogenesis is not completely understood and probably caused by cytokines such as platelet-derived growth factor, β fibroblast–derived growth factor, or transforming growth factor β secreted from clonal megakaryocytes and/or clonal monocyte/histiocyte proliferation [2-4].

Fibrosis grade correlates with other clinical parameters, including hemoglobin, myeloblasts, lactate dehydrogenase, and spleen size [5]. Some studies have found a correlation between grade of fibrosis and survival [5-7]; however, others did not report this correlation [8-11]. BMF is not included in any of the currently used risk classification schemes, including Lille score, Cervantes score, International Prognostic Scoring System (IPSS), Dynamic IPSS, and Dynamic IPSS Plus [12-16]. BMF regression has been reported after allogeneic stem cell transplantation (allo-SCT) [17] and, in some cases, after treatment with IFN-α [18], pomalidomide [19], and, more recently, ruxolitinib [20]; however, the impact of fibrosis resolution—especially the dynamics of resolution—on survival has not been studied to date.

Here we report the impact of dynamic of bone resolution on survival and other graft-specific factors after dose-reduced allo-SCT in a homogenously treated group of patients with advanced primary or post-ET/PV myelofibrosis.

PATIENTS AND METHODS

Between 2002 and 2010, a total of 109 patients underwent allo-SCT for myelofibrosis at the University Medical Center Hamburg-Eppendorf. For inclusion, patients needed to have bone marrow histology investigated by a reference pathologist (M.K., J.T., G.B., or H.K.) before reduced-intensity conditioning allo-SCT and at least on day +30 (±2 weeks) and/or day +100 (±1 month) postengraftment. Fifty-seven patients (median age, 57 years; range, 33-73 years) fulfilled the inclusion criteria. Bone marrow histology was available at both days +30 and +100 in 35 patients, only at day +30 in 13 patients, and only at day +100 in 9 patients. The risk profile was based on IPSS classification [16].

One patient was classified as IPSS low risk; 5, as intermediate-1 risk; 17, intermediate-2 risk, and 34, as high risk. The donor and recipient were related in 11 cases and unrelated in 46 cases, and HLA-matched in 38 cases and HLA-mismatched in 19 cases. The conditioning regimen comprised busulfan 10 mg/kg orally or 10 × 0.8 mg/kg i.v. in combination with fludarabine 150 mg/m^2. Eleven patients received induction therapy with amsacrine, fludarabine, and cytarabine (FLAMSA), followed after a 3-day rest by busulfan/fludarabine. All patients received peripheral blood stem cell grafts. Patient characteristics are summarized in Table 1.

Financial disclosure: See Acknowledgments on page 815.

* Correspondence and reprint requests: Prof Dr Med Nicolaus Kröger, Department for Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Martinistraße 52, D-20246 Hamburg, Germany.
E-mail address: nkroeger@uke.uni-hamburg.de (N. Kröger).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.02.019

Table 1
Patient Charcteristics (n = 57)

Characteristic	Value
Age, yr, median (range)	59 (9-76)
Males/females, n	32/25
Diagnosis, n	
Primary myelofibrosis	41
Post-ET/PV myelofibrosis	16
Donor, n	
Related	11
Unrelated	46
HLA-matched	38
HLA-mismatched	19
IPSS classification at allo-SCT, n	
Low	1
Intermediate-1	5
Intermediate-2	17
High	34
Blasts at allo-SCT, % median (range)	1 (0-17)
Recipient cytomegalovirus serostatus, n	
Positive	38
Negative	19
Conditioning regimen, n	
Busulfan 10 mg/kg/fludarabine 150 mg/kg	46
FLAMSA + busulfan 10 mg/kg/fludarabine 150 mg/kg	11

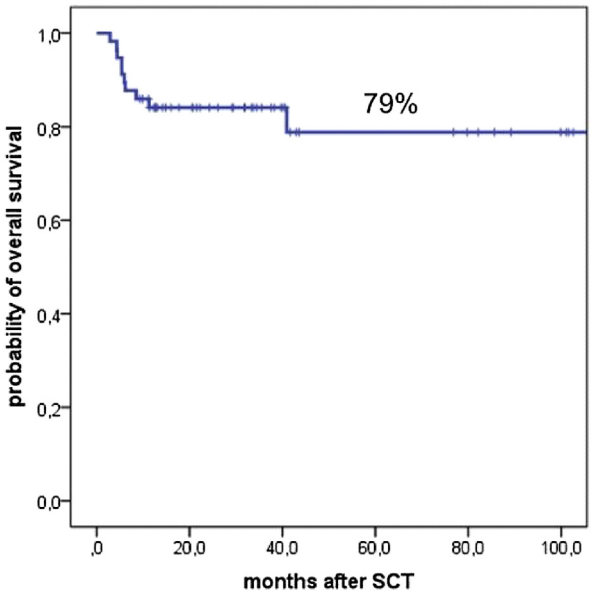

Figure 1. Overall survival after reduced-intensity conditioning for myelofibrosis (n = 57).

BMF was graded according to the European consensus and World Health Organization classification schemes [21]. For this study, only fibrosis regression was evaluated. In cases of residual osteosclerosis but no residual fibrosis, the patient was classified as (MF)-0.

All patients underwent bone marrow investigation before allo-SCT; 48 patients did so again at day +30 and 44 at day +100. Donor cell chimerism and *JAK2V617F* mutation were assessed as decribed previously [22]. Patient care was performed as reported elsewhere [23].

RESULTS

Engraftment and Graft-versus-Host Disease

Three graft failures were observed. All patients received a second donation and successfully engrafted with leukocytes. The median time to leukocyte engraftment ($>1.0 \times 10^9$ cells/L) was 13 days (range, 9-26 days), and the median time to platelet engraftment ($>20 \times 10^9$ cells/L) was 19 days (range, 9-145 days). Acute graft-versus-host disease (GVHD) grade II-IV occurred in 33% and severe grade III-IV GVHD in 14%. Chronic GVHD was seen in 61% of the patients and classified as mild (19%), moderate (33%), or severe (9%). Eleven patients (19%) died during follow-up, either relapse-related (n = 4) or therapy-related (n = 7).

Nonrelapse Mortality, Relapse, and Overall Survival

For the entire study population, nonrelapse mortality at 1 year was 11% (range, 3%-19%). The nonrelapse mortality was calculated from day 0 onward; no patient died before day +30 after allo-SCT, and only 1 patient died between day +30 and day +100. The cumulative incidence of relapse at 3 years after allo-SCT was 20% (range, 4%-36%), and the 5-year estimated overall survival was 79% (range, 65%-93%) (Figure 1). Causes of therapy-related death included GVHD (n = 2), cardiac failure (n = 1), infectious complications (n = 1), organ toxicity (n = 1), liver cirrhosis (n = 1), and secondary graft failure (n = 1).

Dynamic of Fibrosis Regression and Survival

At the time of allo-SCT, 72% of the patients were MF-3 and 28% were MF-2. At day +30 after allo-SCT, 6% were MF-0 and 15% were MF-1, and at day +100, 25% were MF-0 and 29% were MF-1 (Table 2).

Survival of the patients with MF-0/-1 at day +30 was 100%, in contrast to 71% (range, 51%-91%) for those with MF-2/-3 ($P = .10$). Patients with fibrosis regression on day +100 to MF-0/-1 had an 5-year overall survival of 96% (range, 88%-100%), compared with 57% (21%-93%) in those with persistent MF-2/-3 ($P = .04$) (Figure 1). The improved survival of patients with MF-0/-1 at day +100 post–allo-SCT was related to a lower risk of treatment-related mortality (15% versus 4%; $P = .20$) and relapse at 1 year (20% versus 0%; $P = .04$) (Figure 2).

Regarding the fibrosis reduction per level at day +30, 28 (59%) had no reduction, 14 (29%) had a 1-grade reduction, 4 (8%) had a 2-grade reduction, and 2 (4%) had a 3-grade reduction. At day +100 post–allo-SCT, 9 patients (21%) had no reduction in fibrosis grade, whereas 16 patients (36%) had a 1-grade reduction, 12 patients (27%) had a 2-grade reduction, and 7 patients (16%) had a 3-grade reduction (Table 3). Five-year overall survival was improved for patients with a 2- or 3-grade reduction at day +100 in comparison to those with persistent MF-2/-3 (95% [range, 85%-100%] versus 71% [range, 47%-95%]); however, the difference did not reach statistical significance ($P = .19$).

Correlations between Fibrosis Regression and Donor Cell Chimerism, Graft Function, Detection of JAK2V617F Mutation, and IPSS

Comparing the results of fibrosis resolution at day +100 with other disease-specific factors revealed no correlation

Table 2
BMF at allo-SCT and at Day +30 and Day +100 after allo-SCT

Time	BMF, n (%)			
	MF-0	MF-1	MF-2	MF-3
At allo-SCT (n = 57)	0	0	16 (28)	41 (72)
Day +30 (n = 48)	3 (6)	7 (15)	17 (35)	21 (44)
Day +100 (n = 44)	11 (25)	13 (29)	12 (27)	8 (18)

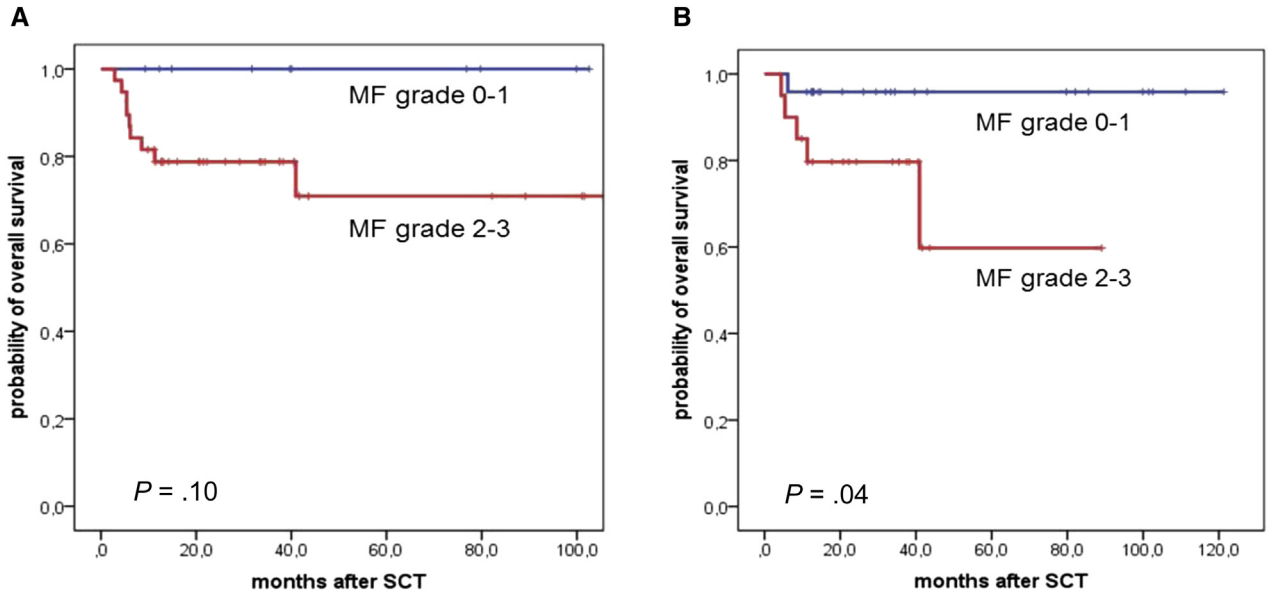

Figure 2. Overall survival according to fibrosis regression on day 30 (A) (based on 48 patient) and day 100 (B) (based on 44 patients) post allografting.

between fibrosis regression and clearance of the *JAK2V617F* mutation in peripheral blood. In 42% of the JAK2-positive patients with MF-0/-1, *JAK2V617F* mutation was still detectable in peripheral blood. In contrast, 81% of the patients with MF-0/-1 had complete donor cell chimerism, compared with only 31% of those with MF-2/-3 (Table 4). There was no significant correlation between BMF resolution on day +100 (MF-2/-3) and IPSS category at time of allo-SCT, suggesting that disease status at transplantation had no significant impact on the dynamic of BMF resolution.

At day +100 post–allo-SCT, 53% of the patients were still RBC transfusion-dependent, and 33% were platelet transfusion–dependent. Patients with MF-2/-3 at day +100 were more likely to be still RBC transfusion–dependent (75% versus 35%; $P = .014$) and platelet transfusion–dependent (50% versus 13%; $P = .018$). The median hemoglobin concentration, leukocyte count, and platelet count on day +100 were higher in patients with MF-0/-1 compared with patients with MF-2/-3 (hemoglobin, 9.8 g/dL versus 8.7 g/dL [$P = .40$]; leukocytes, 3.9×10^9/L versus 3.6×10^9/L [$P = .90$]; platelets, 95×10^9/L versus 55×10^9/L [$P = .30$]), suggesting better (albeit not significantly so) graft function in those patients with rapid resolution of BMF by day +100 (Table 4). There was no difference in acute GVHD grade II-IV between patients with and those without BMF resolution on day +100 (39% versus 30%; $P = .70$). Owing to the low number of events within this study, multivariate analysis could not be performed.

DISCUSSION

This study confirms that complete resolution of BMF occurs in patients with primary and post-ET/PV myelofibrosis after allo-SCT, as previously reported by us and others [24,25]. Furthermore, this study shows for the first time that complete or near-complete resolution, defined as MF-0/-1, on day +100 post–allo-SCT is associated with improved survival. Interestingly, the speed of resolution at day +30 or day +100 was not correlated with the IPSS classification at the time of allo-SCT, confirming that reduced-intensity conditioning can induce as rapid resolution of BMF as has been reported for standard myeloablative conditioning [25].

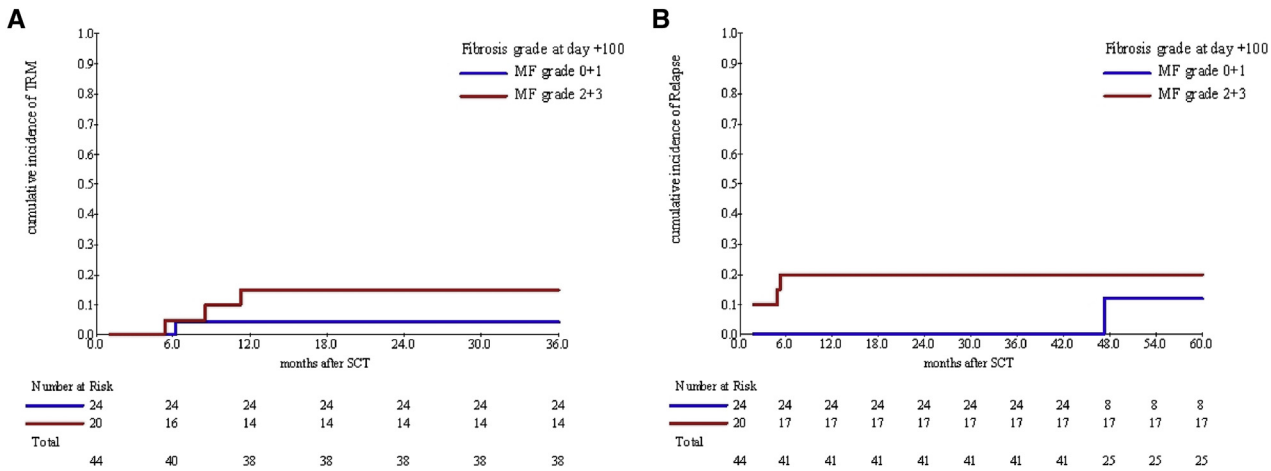

Figure 3. Cumulative incidence of treatment-related mortality (A) and relapse (B) according to BMF regression at day +100 (n = 44).

Table 3
Reduction of BMF at Day +30 and Day +100 after allo-SCT

Time	Level of Reduction, n (%)			
	None	One Grade	Two Grades	Three Grades
Day +30 (n = 48)	28 (59)	14 (29)	4 (8)	2 (4)
Day +100 (n = 44)	9 (21)	16 (36)	12 (27)	7 (16)

Table 4
Correlations between Grade of Fibrosis at Day +100 and *JAK2V617F* Mutation (Only in JAK2-Positive Patients), Donor Cell Chimerism, Graft Function, Risk Status (IPSS), Treatment-Related Mortality, and Overall Survival

Variable	MF-0/-1	MF-2/-3	*P* Value
JAK2V617F mutation, %			.90
Negative	58	54	
Positive	42	46	
IPSS at treatment, %			.50
Low/intermediate-1/-2	60	40	
High	57	43	
Transfusion dependency at day +100, %			
RBCs	35	75	.014
Platelets	13	50	.018
Complete donor cell chimerism, %	81	31	.03
Median hemoglobin at day +100, g/dL	9.8	8.7	.40
Median leukocytes at day +100, $\times 10^9$/L	3.9	3.6	.90
Median platelets at day +100, $\times 10^9$/L	95	55	.30
Treatment-related mortality at 1 yr, %	4	15	.20
Relapse at 1 yr, %	0	20	.04
Five-yr overall survival, %	96	57	.04

Furthermore, there was no correlation between complete resolution of BMF on day +100 and disappearance of the *JAK2V617F* mutant in peripheral blood detected by PCR. Although there was a correlation between donor cell chimerism and fibrosis resolution on day +100, the lack of correlation with *JAK2V617F* mutant level might be explained by the high sensitivity of the assay used in this study [22]. Furthermore, more rapid resolution of BMF was correlated with improved graft function and significantly fewer RBC and platelet transfusions.

Retrospective studies have shown that grade of BMF correlates with disease status [5], but the prognostic impact remains controversial. Most of those previous studies did not identify fibrosis as independent risk factor, and thus none of the currently used risk scores includes BMF. Even if the number of included patients is too small to allow us to draw a meaningful conclusion, this first study on the dynamics of fibrosis regression and its impact on outcome suggests that a more rapid resolution of BMF is associated with improved graft function, less relapse, and improved long-term outcomes in patients with myelofibrosis who undergo allo-SCT. However, this observation will not affect post-transplantation patient management until methods that accelerate BMF regression become available.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

1. Tefferi A, Constantinescu SN. Introduction to "A special spotlight review series on BCR–ABL-negative myeloproliferative neoplasms.". *Leukemia*. 2008;22:3-13.
2. Tefferi A. Myelofibrosis with myeloid metaplasia. *N Engl J Med*. 2000; 342:1255-1266.
3. Chagraoui H, Komura E, Tulliez M, et al. Prominent role of TGF-β1 in thrombopoietin-induced myelofibrosis in mice. *Blood*. 2002;100: 3495-3503.
4. Le Bousse-Kerdilès MC, Martyré MC, Samson M. Cellular and molecular mechanisms underlying bone marrow and liver fibrosis: a review. *Eur Cytokine Netw*. 2008;19:69-80.
5. Thiele J, Kvasnicka HM. Grade of bone marrow fibrosis is associated with relevant hematological findings: a clinicopathological study on 865 patients with chronic idiopathic myelofibrosis. *Ann Hematol*. 2006; 85:226-232.
6. Barosi G, Rosti V, Bonetti E, et al. Evidence that prefibrotic myelofibrosis is aligned along a clinical and biological continuum featuring primary myelofibrosis. *Plos ONE*. 2012;7:e35631.
7. Gianelli U, Vener C, Bossi A, et al. The European consensus on grading of bone marrow fibrosis allows a better prognostication of patients with primary myelofibrosis. *Mod Pathol*. 2013;25: 1193-1202.
8. Nazha A, Estrov Z, Cortes J, et al. Prognostic implications and clinical characteristics associated with bone marrow fibrosis. *Leuk Lymphoma*. 2013;54:2537-2539.
9. Strasser-Weippl K, Steurer M, Kees M, et al. Age and hemoglobin level emerge as most important clinical prognostic parameters in patients with osteomyelofibrosis: introduction of a simplified prognostic score. *Leuk Lymphoma*. 2006;47:441-450.
10. Anger B, Seidler R, Haug U, et al. Idiopathic myelofibrosis: a retrospective study of 103 patients. *Haematologica*. 1990;75:226-234.
11. Alchalby H, Yunus D-R, Zabelina T, et al. Risk models predicting survival after reduced-intensity transplantation for myelofibrosis. *Br J Haematol*. 2012;157:75-85.
12. Cervantes F, Barosi G, Demory JL, et al. Myelofibrosis with myeloid metaplasia in young individuals: disease characteristics, prognostic factors and identification of risk groups. *Br J Haematol*. 1998;102: 684-690.
13. 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.
14. Gangat N, Caramazza D, Vaidya R, et al. DIPSS Plus: a refined Dynamic International Porgnostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. *J Clin Oncol*. 2011;29: 392-397.
15. Passamonti F, Cervantes F, Vannucchi AM, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood*. 2010;115: 1703-1708.
16. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood*. 2009; 113:2895-2901.
17. Thiele J, Kvasnicka HM, Dietrich H, et al. Dynamics of bone marrow changes in patients with chronic idiopathic myelofibrosis following allogeneic stem cell transplantation. *Histol Histopathol*. 2005;20: 879-889.
18. Silver RT, Vadris K, Goldman JJ. Recombinant Interferon-α may retard progression of early primary myelofibrosis: a preliminary report. *Blood*. 2011;117:6669-6672.
19. Tefferi A, Verstovsek S, Barosi G, et al. Pomalidomide is active in the treatment of anemia associated with myelofibrosis. *J Clin Oncol*. 2009; 27:4563-4569.
20. Kvasnicka HM, Thiele J, Bueso-Ramos CE, et al. Exploratory analysis of the effect of ruxolitinib on bone marrow morphology in patients with myelofibrosis. *J Clin Oncol*. 2013;31(Suppl). abstract 7030.
21. Thiele J, Kvasnicka HM, Facchetti F, et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematology*. 2005;90:1128-1133.
22. Kröger N, Badbaran A, Holler E, et al. Monitoring of the JAK2V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. *Blood*. 2007;109:1316-1321.
23. Kröger N, Holler E, Kobbe G, et al. Allogeneic stem cell transplantation after reduced-intensity conditioning in patients with myelofibrosis: a prospective, multicenter study of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Blood*. 2009;114:5264-5270.
24. Kröger N, Thiele J, Zander A, et al., MDS Subcommittee of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Rapid regression of bone marrow fibrosis after dose-reduced allogeneic stem cell transplantation in patients with primary myelofibrosis. *Exp Hematol*. 2007;35:1719-1722.
25. Kerbauy DM, Gooley TA, Sale GE, et al. Hematopoietic cell transplantation as curative therapy for idiopathic myelofibrosis, advanced polycythemia vera, and essential thrombocythemia. *Biol Blood Marrow Transplant*. 2007;13:355-365.