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【 CASE REPORT 】

Salvage Therapy Using Azacitidine for Relapsed Primary Myelofibrosis after Cord Blood Transplantation

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Abstract:
We present the case of a 53-year-old woman with prefibrotic stage primary myelofibrosis (PMF) who underwent cord blood transplantation. Nine years after transplantation, she relapsed, which was confirmed by a bone marrow examination. We decided to treat her using azacitidine. After three courses of azacitidine, a partial cytogenetic response was confirmed. Azacitidine maintenance therapy successfully maintained a low level of recipient-origin peripheral blood cells with a stable hematological condition. Azacitidine may therefore be a promising therapeutic option for PMF patients who relapse after allogeneic stem cell transplantation.

Key words: primary myelofibrosis, allogeneic stem cell transplantation, relapse, azacitidine

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Introduction

Primary myelofibrosis (PMF) is a Philadelphia chromosome-negative myeloproliferative neoplasm (MPN) (1). The *JAK2*-V617F mutation is present in approximately 50-60% of patients with PMF (2). Although the *JAK1/2* inhibitor ruxolitinib is the standard therapy for patients with intermediate- or high-risk PMF, the response to treatment is transient (2). Allogeneic stem cell transplantation (allo-SCT) is the only curative treatment approach for PMF patients. However, disease relapse is one of the common causes of treatment failure after allo-SCT (3-5). There is no standard recommendation regarding the treatment of these patients; an early withdrawal of immunosuppression, the use of donor lymphocyte infusion (DLI) and undergoing a second allo-SCT have been suggested as therapeutic options (6, 7).

Azacitidine (AZA) is a DNA methyltranspherase inhibitor which has been demonstrated to be clinically effective for patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) (8). Moreover, several reports have also suggested that AZA has the potential to alter the donor-induced immunological responses and to be a promising treatment strategy for relapsed allo-SCT recipients with AML or MDS (7, 9, 10). However, there are few studies on AZA therapy for relapsed PMF patients after allo-SCT. We herein report a female PMF patient who relapsed 9 years after cord blood transplantation (CBT) and was successfully treated using AZA.

Case Report

An asymptomatic 53-year-old woman had an abnormal blood count on a routine health care check in 2004. Her white blood cell (WBC) count was 9,200/μL, hemoglobin (Hb) level was 12.4 g/dL and platelet (PLT) count was 85.7 $\times 10^4$/μL. The differential revealed 56% segmented neutrophils, 1% bands, 2% metamyelocytes, 1% myelocytes, 2% eosinophils, 13% basophils, 6% monocytes and 19% lymphocytes. Her physical examination was notable for palpable splenomegaly of 2 cm below the left costal margin. The lactate dehydrogenase (LDH) level increased to 326 U/L (normal range 110-220 U/L). Bone marrow histopathology revealed hypercellularity, increased granulopoiesis and megakaryopoiesis, highly pleomorphic megakaryocytes with atypia, tight clusters of megakaryocytes and grade 1 fibrosis (Fig. 1A, B). A chromosomal analysis demonstrated a normal karyotype. BCR/ABL fusion signal was not detected by fluorescence *in situ* hybridization (FISH) analysis. An analy-

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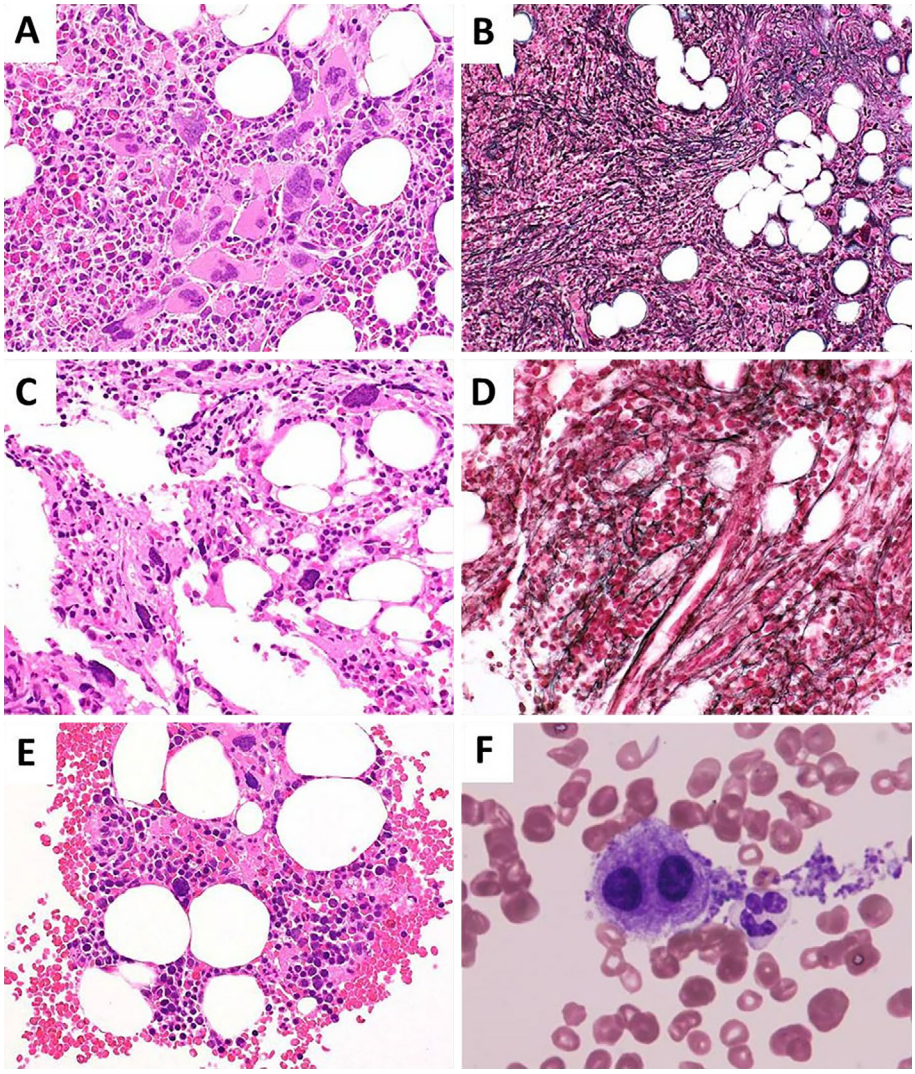

Figure 1. **(A, B) The histological findings of a bone marrow biopsy at diagnosis revealed hypercellularity, increased granulopoiesis and megakaryopoiesis, highly pleomorphic megakaryocytes with atypia, tight clusters of megakaryocytes and grade 1 fibrosis [A: Hematoxylin and Eosin (H&E) staining ×400, B: silver-staining ×400]. (C, D) The histological findings of a bone marrow biopsy at relapse revealed hypercellular marrow with increased megakaryopoiesis without myelofibrosis and highly atypical clusters of megakaryocytes (C: H&E staining ×400, D: silver-staining ×400). (E) The histological findings of a bone marrow clot section at after three courses of azacitidine therapy revealed hypocellular marrow with relative hyperplasia of megakaryopoiesis (H&E staining ×400). (F) A bone marrow aspiration smear at after three courses of azaitidine showed binucleated megakaryocytes (May-Giemsa staining ×600).**

sis for *JAK2*, *CALR* and *MPL* mutations was not performed. Although she was originally diagnosed with essential thrombocytemia, she was recently re-diagnosed with prefibrotic stage PMF according to the 2016 revised World Health Organization (WHO) criteria (1). She was treated following the watchful waiting policy without any thrombotic or hemorrhagic events.

In September 2009, her WBC count was 25,200/μL with 2% myeloblasts, the Hb level was 11.7 g/dL, and the PLT count was 54.3×10^4/μL. The LDH level increased to 462 U/L. Due to disease progression, allo-SCT was considered to be necessary in order to ameliorate the poor prognosis. However, the patient had no human leukocyte antigen (HLA)-matched related donor or matched unrelated donor, and the decision was made to perform CBT. CBT from two-locus HLA-mismatched male umbilical cord blood containing 2.3×10^7 nucleated cells/kg (1.6×10^5 CD34+ cells/kg) was performed in January 2009. Her conditioning regimen consisted of fludarabine at 25 mg/m^2 daily intravenously (i.v.) for 5 consecutive days (total 125 mg/m^2), melpharan at 70 mg/m^2 daily i.v. for 2 days (total 140 mg/m^2) and total body irradiation at 2 Gy twice. Tacrolimus (Tac) was used for prophylaxis against graft-versus-host disease (GVHD). Neutrophil engraftment was achieved on day 25. Grade I acute GVHD of the skin developed on day 70, but resolved spontaneously. Although the patient had limited chronic skin

GVHD, Tac was successfully tapered in January 2010. At this time, the complete blood count consisted of a WBC count of 4,470/μL with a normal differential, Hb level of 12.7 g/dL and PLT count of 16.1×10^4/μL. A sex chromosomal analysis by FISH of peripheral blood nucleated cells revealed full donor male chimerism. Until 2013, full donor chimerism had been confirmed by a sex chromosomal FISH analysis.

In November 2017, the blood count on routine follow-up revealed a high PLT count. The WBC count was 4,230/μL, Hb level was 11.7 g/dL and PLT count was 42.9×10^4/μL. A sex chromosomal analysis by FISH of peripheral blood nucleated cells demonstrated mixed chimerism consisting of 35.7% recipient-origin female karyotype cells and 62.7% donor-origin male karyotype cells. A bone marrow examination revealed hypercellular marrow with 4% myeloblasts without myelofibrosis, increased megakaryopoiesis and clusters of highly atypical megakaryocytes (Fig. 1C, D). No findings of dysplasia were observed in the myeloid or erythroid cells. On a chromosomal analysis, an abnormal karyotype of 46,XX,del(20)(q11.2q13.3)[13]/46,idem,t(X;9)(q11;q34),t(1;4)(p36.1;q21)[3]/46,XY[3] was observed. An analysis of *JAK2*-V617F, *CALR* exon9 and *MPL*-W515L/K mutations was negative. Based on the above, relapse of the primary disease was diagnosed.

In January 2018, the fraction of female karyotype cells and PLT count had increased to 53.7% and 69.7×10^4/μL, respectively. We administered hydroxycarbamide to control the PLT count; however, one month after its initiation, marked anemia was noted (WBC 3,310/μL, Hb 4.7 g/dL and PLT 52.3×10^4/μL). We decided to discontinue hydroxycarbamide therapy and start AZA therapy to control the malignant clone originating from the recipient cells. The patient provided her written informed consent and the use of AZA was approved by our institutional review board. At that time, the patient had limited-type chronic skin GVHD, based on the findings of a skin biopsy.

Considering the risk of myelosuppression, we administered reduced-dose AZA (75 mg/m^2 for 5 days, every 28 days) for disease relapse. Two weeks after the initiation of the first course of AZA therapy, the PLT count decreased to 11.4×10^4/μL, but it re-increased to 52.4×10^4/μL just before the second course. However, the severe anemia was markedly improved by the first course of AZA therapy and the Hb level was stably maintained around the normal range after the second course. After three courses of AZA therapy, a bone marrow examination revealed hypocellular marrow with 0.8% myeloblasts and relative hyperplasia of megakaryopoiesis with atypia (Fig. 1E, F). On a chromosomal analysis, a reduced fraction of recipient-origin female karyotype cells (46,XX,del(20)(q11.2q13.3)[5]/46,idem,t(X;9)(q11;q34),t(1;4)(p36.1;q21)[1]/46,XY[14]) and partial cytogenetic response were confirmed. After 5 courses of AZA therapy, a sex chromosomal analysis by FISH of the peripheral blood nucleated cells demonstrated 2% residual recipient-origin cells. After the sixth course of AZA therapy, we administered full-dose AZA (75 mg/m^2 for 7 days, every 28 days) without any significant adverse events. Although the AZA therapy maintained a low level (0-4%) of recipient-origin cells in the peripheral blood until the 19th course, the fraction of recipient-origin cells increased to 7.5% and 48.0% at the 20th and 24th course of AZA therapy, respectively. From the 22th course, the dose of AZA was reduced to 75 mg/m^2 for 5 days because of grade 3 neutropenia (500/μL). Just before the 24th course of AZA, although grade 3 neutropenia was present, the Hb level and PLT count remained stable. Thus, we decided to continue the administration of AZA as maintenance therapy until aggravation of the hematological condition. During AZA therapy, no deterioration of chronic GVHD was noted. The clinical course after the relapse of primary disease is summarized in Fig. 2.

Discussion

Allo-SCT remains the only curative treatment for myelofibrosis (PMF or post-MPN myelofibrosis) (2-5). The European Society for Blood and Marrow Transplantation (EBMT) reported the outcome of allo-SCT in patients with myelofibrosis (3). The 5-year event-free rate, overall survival rate, and relapse incidence rate were 51%, 67% and 29%, respectively. Therefore, disease relapse remains a significant problem for a successful outcome, and even if the relapse risk decreases over time, it may develop late after transplant. In 2019, the EBMT reported the long-term outcome of allo-SCT in patients with myelofibrosis (5). In this report, the disease-free and overall survival rates at ten years were 32% and 41%, respectively. In the 2-year survivors, the relapse incidence rate was estimated at 21%. Janson et al. reported a poor efficacy of ruxolitinib in relapsed allo-SCT recipients with myelofibrosis (11). In this report, a transient amelioration of the disease-related constitutional symptoms was apparent in some relapsed recipients administered ruxolitinib. However, progressive disease eventually developed in all recipients. Thus, the role of ruxolitinib in allo-SCT remains under debate and the establishment of a treatment strategy for myelofibrosis patients who relapse after allo-SCT is important to overcome this intractable disease.

The EBMT registry reported the outcome of patients with myelofibrosis who relapsed after allo-SCT (6). In this report, the median time to relapse was 7.1 months, with a median overall survival from the time of relapse of 22.9 months. In this cohort, 23% of relapsed patients received DLI alone and 11% underwent chemotherapy alone. The median survival of the DLI alone group was 76 months. On the other hand, the median survival of the chemotherapy alone group was 23 months. In this report, compared with other myeloid malignancies (e.g., AML or high-risk MDS), the prognosis of patients with myelofibrosis relapsing after allo-SCT was relatively good, especially for those who received adoptive immunotherapy. In myelofibrosis patients, allogeneic immunological reaction may be important for eradicating the ma-

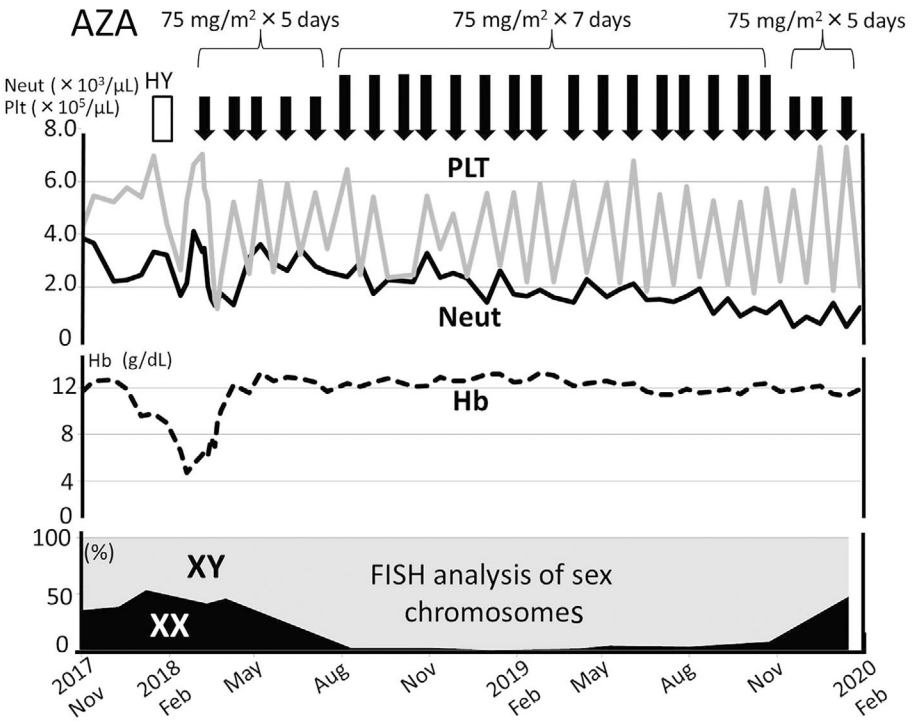

Figure 2. The clinical course after the relapse of primary myelofibrosis. AZA: azacitidine, HY: hydroxycarbamide, Neut: neutrophils, Plt: platelets, Hb: hemoglobin, FISH: fluorescence *in situ* hybridization

lignant clone. However, as our patient received cord blood as the graft source, we were unable to select a DLI for her relapsed disease.

This is, to our knowledge, the first report of a CBT recipient with relapsed PMF who was successfully treated using AZA. However, AZA may have limited efficacy in non-transplant patients with PMF or post-MPN myelofibrosis regardless of the induction of global hypomethylation (12). Andriani et al. reported the results of AZA therapy that was administered to 39 advanced MPN patients, including 13 PMF patients, other than allo-SCT recipients (13). In the report, 17.5% and 20.5% of the entire cohort achieved a partial response (PR) and a complete response (CR) respectively. However, the treatment results of AZA for 13 PMF patients were not detailed in this report. Quintás-Cardama et al. reported a phase II study of AZA for 34 patients with PMF and post-MPN myelofibrosis (12). In this report, although no patients achieved CR, 1 patient achieved PR and a clinical improvement was noted in 7. Badar et al. reported the efficacy of another hypomethylating agent, decitabine, in 11 high-risk PMF patients. Nine patients benefitted from decitabine, but none achieved CR or PR (14). On the other hand, several studies have reported that AZA can induce remission in AML or MDS patients who relapse after allo-SCT (7, 9, 10). Craddock et al. reported that 46 (CR: n=24, PR: n=22) out of 157 AML patients who relapsed after allo-SCT responded to AZA therapy (9). In this report, the 2-year survival rate of patients who achieved CR was 48% versus 12% for the entire population. However, there is little information about the efficacy of AZA in myelofibrosis patients who relapse after allo-SCT. Goodyear et al. reported that AZA can up-regulate the expression of epigenetically silenced tumor antigens and induce CD8+ T-cell responses to tumor antigens after transplant (15). Ishikawa et al. reported relapsed MDS recipients who were successfully treated by graft-versus-leukemia effects with evident WT1-specific T-cell responses, which were induced by AZA and DLI (16). In our patient, these immunological effects may have played an important role in the response to AZA therapy.

Although the majority of PMF patients have mutations in *JAK2*-V617F, *CALR* or *MPL* genes, up to 10% are negative for these mutations (1). Tefferi et al. reported that the triple-negative PMF (TN-PMF) patients had a poorer prognosis than *JAK2*-mutated, *MPL*-mutated or *CALR*-mutated PMF patients (median survival; 2.5 years, 4.3 years, 4.1 years and 8.2 years, respectively) (17). Andriani et al. reported that the *JAK2*-V617F mutation was not associated with a better response rate in advanced MPN patients treated using AZA (13). On the other hand, Quintás-Cardama et al. noted responses to AZA therapy among myelofibrosis patients with poor-risk cytogenetic factors and those carrying *JAK2*-V617F (12). Although TN-PMF is currently considered to comprise a molecularly and clinically heterogeneous disease group, adverse prognostic relevance has been demonstrated for certain mutations, including *ASXL1* and *SRSF2* (2, 17). The absence of driver mutations makes it difficult to distinguish PMF from other myeloid malignancies with associated bone marrow fibrosis (18). According to Martín et al., molecular genetics are useful to predict responses to AZA therapy by MDS or AML patients (19). Patients with *SF3B1*

mutations had a better response to AZA. In contrast, patients with mutations in *RUNX1, SETBP1 or NPM1* had a poorer response. Kröger et al. reported the impact of molecular genetics on the outcome after allo-SCT in myelofibrosis recipients. In this report, the *CALR* mutation was an independent factor for a favorable outcome. On the other hand, *ASXL1* and *IDH2* mutations were independent risk factors for an unfavorable prognosis (20). In our patient, a molecular genetic analysis, other than for driver mutations, was not performed. However, a molecular genetic analysis may be useful to predict not only the outcome of allo-SCT, but also the responses to AZA therapy by PMF patients.

Conclusion

Although allo-SCT is the only curative treatment for PMF, disease relapse remains a significant problem for this intractable disease (2). Malignant clones in PMF patients may be more susceptible to immunological intervention (e.g. DLI, second allo-SCT) than AML or MDS (6). However, CBT recipients cannot receive DLI for relapsed disease. Several studies demonstrated that AZA has not only direct anti-tumor effects, but also potent immunological effects to alter graft-versus-leukemia effects in relapsed allo-SCT recipients with AML or MDS (15, 16). Therefore, AZA may be a promising treatment strategy for PMF patients who relapse after allo-SCT.

The authors state that they have no Conflict of Interest (COI).

References

1. Thiele J, Barbui T, Kvasnicka HM, et al. Primary myelofibrosis. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th ed. Swerdlow SH, Campo E, Harris NL, et al., Eds. IARC Press, Lyon, 2017: 44-50.
2. Tefferi A. Primary myelofibrosis: 2017 update on diagnosis, risk-stratification, and management. Am J Hematol **91**: 1262-1271, 2016.
3. 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 **114**: 5264-5270, 2009.
4. Ballen KK, Shrestha S, Sobocinski KA, et al. Outcome of transplantation for myelofibrosis. Biol Blood Marrow Transplant **16**: 358-367, 2010.
5. Robin M, de Wreede LC, Wolschke C, et al. Long-term outcome after allogeneic hematopoietic cell transplantation for myelofibrosis. Haematologica **104**: 1782-1788, 2019.
6. McLornan DP, Szydlo R, Robin M, et al. Outcome of patients with Myelofibrosis relapsing after allogeneic stem cell transplant: a retrospective study by the Chronic Malignancies Working Party of EBMT. Br J Haematol **182**: 418-422, 2018.
7. Schuler E, Boughoufala S, Rautenberg C, et al. Relapse patterns and treatment strategies in patients receiving allogeneic hematopoietic stem cell transplantation for myeloid malignancies. Ann Hematol **98**: 1225-1235, 2019.
8. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol **10**: 223-232, 2009.
9. Craddock C, Labopin M, Robin M, et al. Clinical activity of azacitidine in patients who relapse after allogeneic stem cell transplantation for acute myeloid leukemia. Haematologica **101**: 879-883, 2016.
10. Woo J, Deeg HJ, Storer B, et al. Factors determining responses to azacitidine in patients with myelodysplastic syndromes and acute myeloid leukemia with early post-transplantation relapse: a prospective trial. Biol Blood Marrow Transplant **23**: 176-179, 2017.
11. Janson D, Ayuk FA, Wolschke C, et al. Ruxolitinib for myelofibrosis patients relapsing after allogeneic hematopoietic transplantation [Abstract]. Blood **128**: 1948, 2016.
12. Quintás-Cardama A, Tong W, Kantarjian H, et al. A phase II study of 5-azacitidine for patients with primary and post-essential thrombocythemia/polycythemia vera myelofibrosis. Leukemia **22**: 965-970, 2008.
13. Andriani A, Elli E, Trapè G, et al. Treatment of Philadelphia-negative myeloproliferative neoplasms in accelerated/blastic phase with azacitidine. Clinical results and identification of prognostic factors. Hematol Oncol **37**: 291-295, 2019.
14. Badar T, Kantarjian HM, Ravandi F, et al. Therapeutic benefit of decitabine, a hypomethylating agent, in patients with high-risk primary myelofibrosis and myeloproliferative neoplasm in accelerated or blastic/acute myeloid leukemia phase. Leuk Res **39**: 950-956, 2015.
15. Goodyear OC, Dennis M, Jilani NY, et al. Azacitidine augments expansion of regulatory T cells after allogeneic stem cell transplantation in patients with acute myeloid leukemia (AML). Blood **119**: 3361-3369, 2012.
16. Ishikawa T, Fujii N, Imada M, et al. Graft-versus-leukemia effect with a WT1-specific T-cell response induced by azacitidine and donor lymphocyte infusions after allogeneic hematopoietic stem cell transplantation. Cytotherapy **19**: 514-520, 2017.
17. Tefferi A, Lasho TL, Finke CM, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia **28**: 1472-1477, 2014.
18. Alvarez-Larrán A, López-Guerra M, Rozman M, et al. Genomic characterization in triple-negative primary myelofibrosis and other myeloid neoplasms with bone marrow fibrosis. Ann Hematol **98**: 2319-2328, 2019.
19. Martín I, Navarro B, Serrano A, et al. Impact of clinical features, cytogenetics, genetic mutations, and methylation dynamics of CDKN2B and DLC-1 promoters on treatment response to azacitidine. Ann Hematol **99**: 527-537, 2020.
20. Kröger N, Panagiota V, Badbaran A, et al. Impact of molecular genetics on outcome in myelofibrosis patients after allogeneic stem cell transplantation. Biol Blood Marrow Transplant **23**: 1095-1101, 2017.