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 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 primary myelofibrosis 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 transplant (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 methyltransferase 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 ×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). 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 (Figure A, B). A chromosomal analysis demonstrated a nor-
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 azacitidine showed binucleated megakaryocytes (May-Giems staining ×600).

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 (1.6×10^7 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 spon-

mal karyotype. BCR/ABL fusion signal was not detected by fluorescence in situ hybridization (FISH) analysis. An analysis for JAK2, CALR and MPL mutations was not performed. Although she was originally diagnosed with essential thrombocytopenia, she was recently re-diagnosed with prefibrotic stage PMF according to the 2016 revised 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 54.3×10^3/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.
taneously. 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 (Figure C, 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-W515 L/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 hydroxyurea 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 hydroxyurea 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 adminis-
tered 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 (Figure E, 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)(p36.1q21)[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 Figure 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 immu-
nological reaction may be important for eradicating the malignant 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 ther-

**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
apy 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 in relapsed allo-SCT recipients with AML or MDS (15, 16). Therefore, AZA may be more susceptible to immunological intervention (e.g. CDKN2B and DLC-1 promoters) on treatment response to azacitidine and donor lymphocyte infusions after allogenic hematopoietic stem cell transplantation. A Prospective Trial. Biol Blood Marrow Transplant 23: 176-179, 2017.

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

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