Donor-derived diffuse large B-cell lymphoma after haploidentical stem cell transplantation for acute myeloid leukemia

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We report a case of donor-derived diffuse large B-cell lymphoma (DLBCL), which developed 5 years after stem cell transplantation from a human leukocyte antigen (HLA)-haploidentical donor for acute myeloid leukemia (AML). A 51-year-old male was diagnosed with AML with variant KMT2A translocation involving t(6;11)(q13;q23). After 12 cycles of azacitidine treatment, fluorescence in situ hybridization (FISH) for KMT2A split signal indicated that 94% of his bone marrow (BM) cells were positive. He underwent peripheral blood stem cell transplantation (PBSCT) from his HLA-haploidentical son. The preconditioning regimen consisted of fludarabine, busulfan, melphalan, and antithymocyte globulin (ATG). The graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and short-term methotrexate. On day 28, KMT2A FISH analysis indicated that he had achieved a complete response (CR). He continued to receive tacrolimus for the limited type of cutaneous chronic GVHD. Five years after the transplantation, positron emission tomography/computed tomography (PET/CT) showed an abdominal tumor. The tumor was diagnosed as DLBCL without Epstein-Barr virus. BM aspiration revealed the infiltration of lymphoma cells with t(8;14)(q24;q32). Chimerism analysis showed that both the peripheral blood (PB) and abdominal lymphoma cells were of donor origin. After 4 cycles of salvage chemotherapy, PET/CT showed that a CR had been achieved. He underwent a second PBSCT from an HLA-identical unrelated donor. The preconditioning regimen and GVHD prophylaxis were the same as those for the first PBSCT without ATG. The patient’s PB revealed complete second donor-type chimerism, and the patient has maintained a CR since the second transplantation.

Keywords: post-transplantation lymphoproliferative disorder (PTLD), haploidentical stem cell transplantation, chronic GVHD

INTRODUCTION

Advances in hematopoietic stem cell transplantation (HSCT) have led to an increasing number of transplant survivors.1 One of the long-term complications associated with allogeneic HSCT is the development of secondary malignancies.2 Secondary malignancies can be separated into three types. The first is post-transplantation lymphoproliferative disease (PTLD), which is related to a compromised immune status and Epstein-Barr virus (EBV) infections. PTLD is mainly donor-derived, and the median time to the development of EBV-PTLD after HSCT is 2–4 months.3 The second type is myelodysplastic syndrome (MDS) and acute leukemia, which occurs one to three years after transplantation, and the third type is solid tumors, which increase in incidence over time.4 The incidence of secondary solid malignancies can continue to rise over time, and studies with follow-up periods of up to 20 years have not shown a plateau in their occurrence.5

Here, we report a case of donor-derived diffuse large B-cell lymphoma (DLBCL) without EBV, which developed 5 years after the patient underwent allogeneic transplantation for acute myeloid leukemia (AML) from a human leukocyte antigen (HLA)-haploidentical donor. The lymphoma behaved as if it was a secondary solid tumor that had arisen after HSCT. The patient was successfully treated with salvage chemotherapy and a second allogeneic transplantation from another HLA-identical unrelated donor.

CASE REPORT

A 51-year-old male visited another hospital regularly for...
the treatment of hypertension and hyperlipidemia. He had exhibited progressive pancytopenia for 8 months. His laboratory findings demonstrated a white blood cell (WBC) count of 900/μL (blasts: 3%, metamyelocytes: 1%, bands: 2%, polymorphonuclear leukocytes: 60%, monocytes: 4%, lymphocytes: 30%, 19 erythroblasts/100 WBC), a hemoglobin level of 10.1 g/dL, and a platelet count of 4.8×10^4/μL. Bone marrow (BM) aspiration showed a myeloblast frequency of 16.8% and an erythroblast frequency of 65.2% with pseudo Pelger–Huët cells and micromegakaryocytes (Figures 1A and 1B). Flow cytometry indicated that the myeloblasts were positive for CD13, CD33, CD34 (weakly), and HLA-DR. G-band analysis showed 18 t(6;11)(q13;q23) translocations among the 20 analyzed cells. The patient was diagnosed with AML with variant KMT2A (previously called MLL) translocation according to the 2016 revision of the WHO classification.

After 8 cycles of azacitidine (75 mg/m² of azacitidine for 7 consecutive days every 28 days), he did not need a red blood cell transfusion. After 12 cycles of azacitidine, his BM had a myeloblast frequency of 2.0%; however, the t(6;11)(q13;q32) translocation was detected in 17 of 20 cells during G-band analysis, and fluorescence in situ hybridization (FISH) analysis for KMT2A split signal showed that 94% of his BM cells were positive. We planned to treat him with allogeneic HSCT. We chose his 19-year-old son, who had haploidentical HLA, as a donor. The patient had no HLA antibodies. The interval from diagnosis to transplantation was 12 months. The patient’s hematopoietic cell transplantation (HCT)-specific comorbidity index (HCT-CI) score was 0.7. Our haploidentical transplantation procedure does not include post-transplantation cyclophosphamide for graft-versus-host disease (GVHD) prophylaxis, but instead employs a method that was previously reported by Hyogo

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**Fig. 1.** (A), (B) BM smears obtained at the diagnosis of AML showed erythroid proliferation with myeloblasts (arrows) and micromegakaryocytes (arrowhead). (C) A tumor biopsy performed at the diagnosis of DLBCL showed diffuse proliferation of large lymphoma cells with many tingible body macrophages. (D) CD20 staining, (E) BCL6 staining, (F) MYC staining. (G) BM smear at the diagnosis of DLBCL revealed the infiltration of atypical large lymphoid cells.
College of Medicine in Japan. In this approach, patients are infused with unmanipulated stem cells from a haploidentical donor using fludarabine, busulfan, low-dose antithymocyte globulin (ATG), and steroids. In this case, the patient received a non-myeloablative (reduced-intensity) preconditioning regimen, which consisted of 30 mg/m² of fludarabine for 6 days (day -7 to day -2), 3.2 mg/kg/day of intravenous busulfan for 2 days (day -5 to day -4), 50 mg/m² of melphalan for 2 days (day -3 to day -2), and 2.5 mg/kg of rabbit ATG (Thymoglobuline) for 1 day (day -2), as described previously. At the age of 52, he was infused with donor peripheral blood stem cells (PBSC) (4.78×10⁶ CD34+ cells/kg). Tacrolimus (TAC), which was administered via a continuous infusion, was initiated on the day before the transplantation at a dose of 0.02 mg/kg/day. Neutrophil and platelet engraftment occurred on days 10 and 19, respectively. The patient achieved a complete response (CR) on day 28 because his BM showed a normal karyotype during G-banding analysis, and FISH analysis indicated that his BM was negative for KMT2A. On days 28 and 123, his PB revealed complete donor-type chimerism during variable number tandem repeat (VNTR) analysis. He developed adenovirus hemorrhagic cystitis on day 19, which resolved spontaneously. He did not receive any cytomegalovirus (CMV) prophylaxis. CMV antigenemia was diagnosed on day 42, and he was successfully treated with ganciclovir. On day 13, small eruptions spread over his trunk and extremities. He was diagnosed with acute GVHD (aGVHD) based on a skin biopsy. The grade of aGVHD was II (skin stage: 3, liver stage: 0, gut stage: 0), and he was treated with prednisolone. He continued to have limited skin lesions and was diagnosed with the progressive type of chronic GVHD (cGVHD) based on a skin biopsy on day 125. As the limited type of cGVHD persisted, he was not able to stop receiving the TAC treatment.

Five years after the transplantation, his lactate dehydrogenase (LDH) level increased from 302 U/L to 3,320 U/L for 3 months (Table 1). Although he had no symptoms, 18F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) showed an abdominal tumor (maximum standardized uptake value [SUVmax]: 21.1) and pelvic bone uptake (SUVmax: 14.1) (Figure 2A). An abdominal biopsy specimen demonstrated diffuse proliferation of large atypical lymphoid cells (Figure 1C). These cells were immunohistochemically positive for CD10 (weak), CD20 (Figure 1D), CD79a, and BCL6 (Figure 1E), and negative for

| Table 1. Laboratory findings at the onset of lymphoma |
|-----------------------------------------------------|
| Peripheral blood                                    | Bone marrow                              |
| WBC 4300 /mL                                        | Atypical cells                           |
| seg 75 %                                            | G-banding                               |
| eos 3 %                                             | 59<2p>, XY, +X, +1, +5, +6, +7, t(8;14)(q24;q32), |
| mo 11 %                                             | +11,+12,+12,+16,+17,+20,+21,+mar1 [8] |
| lym 11 %                                            | 46, XY [12]                             |
| RBC 432 x10⁶/mL                                     | IGH-MYC (FISH) 5%                        |
| Hb 13.9 g/dL                                        | KMT2A (FISH) 0%                          |
| Ht 41.1 %                                           |                                          |
| Ptt 23.0 x10⁶/mL                                    |                                          |
| PT-INR 0.97                                        |                                          |
| APTT 31.8 sec                                       |                                          |
| Fbg 415 mg/dL                                      |                                          |
| FDP 6.2 mg/mL                                      |                                          |
| DD 3.1 mg/mL                                       |                                          |
| Cr 0.77 mg/dL                                      |                                          |
| TP 7.0 g/dL                                         |                                          |
| TP 7.0 g/dL                                         |                                          |
| ALB 3.4 g/dL                                       |                                          |
| AST 27 U/L                                          |                                          |
| ALT 22 U/L                                         |                                          |
| ALP 157 U/L                                        |                                          |
| LDH 3320 U/L                                       |                                          |
| γ-GTP 33 U/L                                       |                                          |
| UA 7.5 mg/dL                                        |                                          |
| Na 138 mEq/L                                       |                                          |
| K 4.3 mEq/L                                        |                                          |
| Cl 101 mEq/L                                       |                                          |
| Ca 8.9 mg/dL                                        |                                          |
| Glu 149 mg/dL                                      |                                          |
| HbA1c 6.7 %                                        |                                          |
| CRP 10.75 mg/dL                                    |                                          |
| IgG 1309 mg/dL                                     |                                          |
| IgA 246 mg/dL                                      |                                          |
| IgM 140 mg/dL                                      |                                          |
| Ferritin 1057.8 ng/mL                              |                                          |
| β2MG 2.9 mg/L                                      |                                          |
| sIL-2R 794 U/mL                                    |                                          |
| HTLV-1 (-)                                          |                                          |
| EBV                                                |                                          |
| VCA-IgG x640                                       |                                          |
| EBNA x80                                            |                                          |
CD3, CD5, BCL2, and MUM1. Immunohistochemical staining showed that 60% of the tumors cells were positive for MYC (Figure 1F) and 90% were positive for Ki-67, whereas in situ hybridization with EBV-encoded small nuclear early region 1 (EBER 1 ISH) produced negative results. Flow cytometry demonstrated that the tumor cells were positive for CD19, CD20, CD21, CD22, the λ light chain, CD38, FMC-7, and HLA-DR. FISH analysis produced the following results: IGH/MYC rearrangement: 62%, IGH/BCL2 rearrangement: 0% and BCL6 split signal: 0%. No G-banding results were obtained because of insufficient proliferation. He was diagnosed with DLBCL, and the tumors were classified into germinal center B cell–like (GCB) subtype according to Hans classification. His DLBCL was also categorized in monomorphic PTLD by WHO classification. VNTR analysis showed that both the PB and abdominal lymphoma cells were of donor origin. BM aspiration revealed the infiltration of lymphoma cells (frequency: 0.4%) (Figure 1G), G-banding analysis showed complicated abnormalities including t(8;14)(q24;q32), and FISH analysis revealed an IGH/MYC rearrangement frequency of 5% and was negative for KMT2A (Table 1).

The patient was diagnosed with donor-derived DLBCL. The disease was categorized as stage IV according to the Lugano classification, as it had caused an abdominal tumor and affected the BM and pelvic bone. He was classified as low-intermediate risk according to the International Prognostic Index (IPI) and as high-intermediate risk according to the National Comprehensive Cancer Network (NCCN)-IPI. At that time, the donor, his son, was 25 years old and was living a completely healthy life. The TAC treatment was discontinued immediately, and the patient was treated with the dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (DA-EPOCH-R) regimen. After one course of DA-EPOCH-R, his LDH level decreased to 219 U/L. After 4 cycles of DA-EPOCH-R, he was confirmed to be in metabolic CR by PET/CT (Figure 2B), his BM showed a normal karyotype during G-banding analysis, and FISH analysis demonstrated that he was negative for IGH/MYC. He was scheduled to receive a second allogeneic transplantation from an HLA-identical unrelated male donor. The interval from diagnosis to transplantation was 4 months. His HCT-CI score was 0. The conditioning regimen and GVHD prophylaxis were the same as those used for the first transplantation, although ATG was not used. At the age of 57, he was transplanted with donor PBSC (5.74×10^6 CD34+ cells/kg). Neutrophil and platelet engraftment occurred on days 12 and 19, respectively. On days 30 and 97, his PB showed complete second donor-type chimerism during VNTR analysis. He developed adenovirus hemorrhagic cystitis again on day 30, which resolved spontaneously. He received letermovir prophylaxis until day 100, and he did not develop CMV antigenemia. Although he did not develop aGVHD, he suffered from an erythematous rash on day 111. He was diagnosed with extensive de novo cGVHD based on a skin biopsy. The addition of prednisone to TAC was not sufficient to regulate the symptoms of cGVHD. He was successfully treated with 420 mg of daily ibrutinib, which is an inhibitor of Bruton tyrosine kinase that is effective against cGVHD. He continued to maintain a CR one year after the second allogeneic HSCT.

Fig. 2. (A) ^18F-FDG PET/CT performed at the diagnosis of DLBCL showed an abdominal tumor, and (B) the abnormal ^18F-FDG uptake disappeared after 4 cycles of DA-EPOCH-R treatment.
DISCUSSION

A large number of HSCT recipients achieve long-term survival, and more attention should be given to their quality of life and late complications. Secondary malignancies are an important late complication of HSCT. Donor cell-derived leukemia/lymphoma (DCL) is an extremely rare outcome after allogeneic HSCT. Three previous large surveys have assessed the frequency of DCL. The European Group for Blood and Marrow Transplantation identified 14 DCL cases among 10,489 allogeneic HSCT,17 and the University of Minnesota reported 8 DCL cases among 2,390 allogeneic HSCT.18 In addition, a Japanese survey identified 40 DCL cases among 36,870 allogeneic HSCT, and the incidence of DCL was estimated to be 0.16% at 15 years. Although 5 cases of non-Hodgkin lymphoma were identified in 40 DCL cases, the histological types were not shown.19 B-cell PTLD that arises after allogeneic HSCT is usually diagnosed within 3 months, and it is almost always of donor origin and associated with EBV-genomic DNA integration.4 Our case involved late-onset DLBCL; i.e., it developed 5 years after the first allogeneic HSCT. Although the level of EBV-DNA in the patient’s PB was not evaluated, his lymphoma was not related to EBV reactivation because his tumor was negative for EBER 1 ISH. Previously, 33 cases of post-transplantation DLBL (PT-DLBL) were reported.20 EBV-positivity was 72%. The median interval between transplantation and diagnosis of EBV(+) and EBV(-) PT-DLBL were 1.15 years and 18 years, respectively. All EBV(+) PT-DLBL cases were of activated B cell (ABC) origin, whereas 45% EBV(-) PT-DLBL cases were of GCB origin. The authors showed that EBV(+) and EBV(-) PT-DLBL have distinct gene expression profiles, and the transcriptomic profile of EBV(-) PT-DLBL was similar to that of DLBCL in immunocompetent individuals. Our patient still needed TAC therapy was necessary to achieve long-term remission. At the diagnosis of DLBCL in our case, which occurred 5 years after the first transplantation, the patient’s PB revealed complete first donor-type chimerism, and FISH analysis of his BM showed that it was negative for KMT2A. Therefore, although the haploidentical donor immune cells contributed to the cGVHD, they may also have prevented the recurrence of AML with variant KMT2A translocation.

In conclusion, we diagnosed a patient with donor-derived DLBCL 5 years after a haploidentical transplantation for AML. Although his lymphoma was high-grade and exhibited rapid growth, he was successfully treated with salvage chemotherapy and a second transplantation from another unrelated donor. He developed cGVHD skin lesions, but ibrutinib was effective at controlling them. In addition, it is suspected that ibrutinib not only suppressed the development of cGVHD, but also prevented any relapse of the B-cell lymphoma. The patient has been in CR for one year since the second allogeneic HSCT; however, long-term follow-up will be needed to assess the outcomes of the second transplantation.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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