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CASE REPORT

Pediatric post-transplant diffuse large B cell lymphoma after cardiac transplantation

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Abstract Post-transplant lymphoproliferative disorders (PTLDs) occur in 3.5–9% of patients after pediatric cardiac transplantation. Caution is needed when treating patients with PTLD because of the risk of allograft rejection frequently caused by withdrawal of immunosuppression. In this report, we describe a 47-month-old boy who developed PTLD as an ileocecal mass 29 months after cardiac transplantation. Immunosuppressive therapy with cyclosporine A (CyA) had been reduced due to an elevation of Epstein-Barr virus (EBV) titer for 8 months before development of PTLD. Histology of the tumor was diffuse large B cell lymphoma. EBV was detected by in situ hybridization assay. Cytogenetic analysis revealed *t*(8;14)(q24;q32) and Southern blot analysis detected a *c-Myc* rearrangement. He was treated with rituximab and combination chemotherapy with excellent response. CyA dose was maintained at reduced levels during chemotherapy and later minimized with introduction of everolimus. The child is free of both PTLD and allograft rejection 41 months after the diagnosis of PTLD.

Keywords Cardiac transplantation · Diffuse large B cell lymphoma · Pediatric · Post-transplant lymphoproliferative disorder

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1 Introduction

Post-transplant lymphoproliferative disorders (PTLDs) are an important cause of morbidity and mortality after pediatric cardiac transplantation. Although its pathological range is quite diverse, from monomorphic to polymorphic proliferation, the majority are B lymphocyte disorders and associated with Epstein-Barr virus (EBV) [1, 2]. Withdrawal of immunosuppression for management of PTLD can often lead to allograft rejection and transplant coronary artery disease [1]. We report a child with EBV-associated post-transplant diffuse large B cell lymphoma (DLBL) after cardiac transplantation who was successfully treated with rituximab and combination chemotherapy.

2 Case report

A 17-month-old boy underwent successful cardiac transplantation for dilated cardiomyopathy in October 2002. He received cyclosporine A (CyA), initially with azathioprine and later with mycofenolate mofetil for post-transplant immunosuppression. He was EBV-seronegative pre-transplant (VCA-IgG: <tenfold) and his donor was EBV-seropositive pre-transplant (VCA-IgG: 320-fold). In November 2003, his EBV antibody titer for VCA-IgG increased to 2,560-fold. In August 2004, 21 months

post-transplant, CyA dose was reduced and mycophenolate mofetil was stopped due to an increase in EBV titers (92,813 copies/DNA 1 μg; Fig. 1); no lymph node swelling or fever was observed; and no abnormalities were detected on CT scan or gallium scintigraphy. He also received ganciclovir or valganciclovir, and IV immunoglobulin with high titer of antibodies against EBV to prevent the development of PTLD. In April 2005, at 47 months of age and 29 months post-transplant, he developed abdominal pain. CT scan revealed an ileocecal mass (Fig. 2a). An open biopsy of the tumor established a diagnosis of monomorphic PTLD, DLBL (Fig. 3a). Immunoblasts were $CD20^+$ (Fig. 3b), $CD79a^+$, and $CD3^-$. In situ hybridization for EBV early RNA (EBER) showed reactivity in lymphoid cells (Fig. 3c). Conventional cytogenetic analysis revealed 46, XY, *t*(8;14)(q24;q32). Southern blot analysis detected a *c-Myc* rearrangement in tumor cells in ascites (Fig. 4). At this point, EBV titer was increased to 15,000 copies/10^6 WBCs (normal value <20 copies). EBV antibody titers for VCA-IgG, VCA-IgM, EADR-IgG, and EBNA were 1,280-fold, <10-fold, <10-fold, and 40-fold, respectively. Laboratory studies showed a WBC count of 4,470 μL^{-1}, hemoglobin 9.9 g/dL, lactate dehydrogenase 1,261 IU/L, serum soluble interleukin-2 receptor (sIL-2R) level 1,947 U/mL (normal value 150–505 U/mL), and normal electrolytes and liver function.

The clinical course of this patient is summarized on Fig. 1. Immunosuppressive therapy with oral CyA was continued to maintain trough levels at 50–90 ng/mL. He received six courses of weekly rituximab (375 mg/m^2) with one course of cyclophosphamide (600 mg/m^2), and seven courses of combination chemotherapy. Combination chemotherapy was as follows: regimen A consisted of high-dose methotrexate, vincristine, cyclophosphamide, pirarubicin (THP-adriamycin), and dexamethasone; regimen B, methotrexate and cytosine arabinoside; and regimen C, cytosine arabinoside and etoposide. Excellent response was observed with resolution of tumor mass (Fig. 2b) and reduction of EBV titer (Fig. 1). Serum sIL-2R level decreased to 987 U/mL 2 weeks after the start of chemotherapy. After completion of chemotherapy, oral everolimus was started, followed by a further reduction of oral CyA to maintain trough levels at 30–60 ng/mL. EBV titer was moderately increased up to 1,000 copies/10^6 WBCs and serum sIL-2R level was maintained at <1,000 U/mL. He remains in complete remission 41 months following diagnosis of DLBL without allograft rejection.

3 Discussion

The incidence of PTLD has been reported to range from 3.5 to 9% after pediatric cardiac transplantation [1–4]. PTLD comprises two pathological types, i.e., polymorphic and monomorphic. Early-onset disease (≤3 years post-transplant) is frequently observed with polymorphic localized disease, while late-onset disease (>3 years post-transplant) is more often associated with monomorphic disseminated disease [1, 5]. Most cases were of B cell

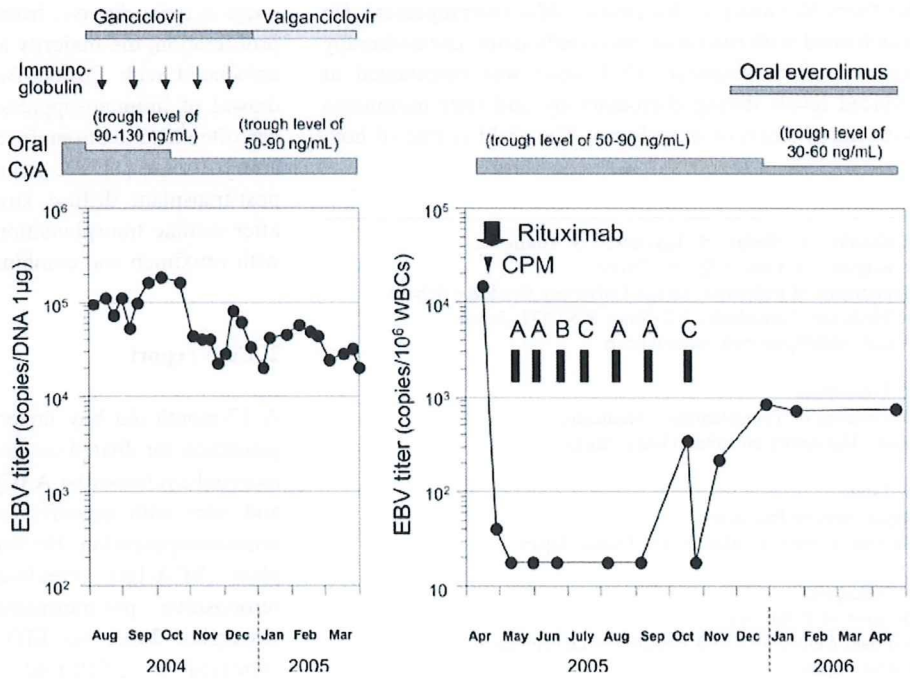

Fig. 1 Clinical course and changes in EBV titer. *CyA* cyclosporine A, *CPM* cyclophosphamide, *EBV* Epstein-Barr virus. **a**–**c** indicate combination chemotherapy regimens (details are shown in the text)

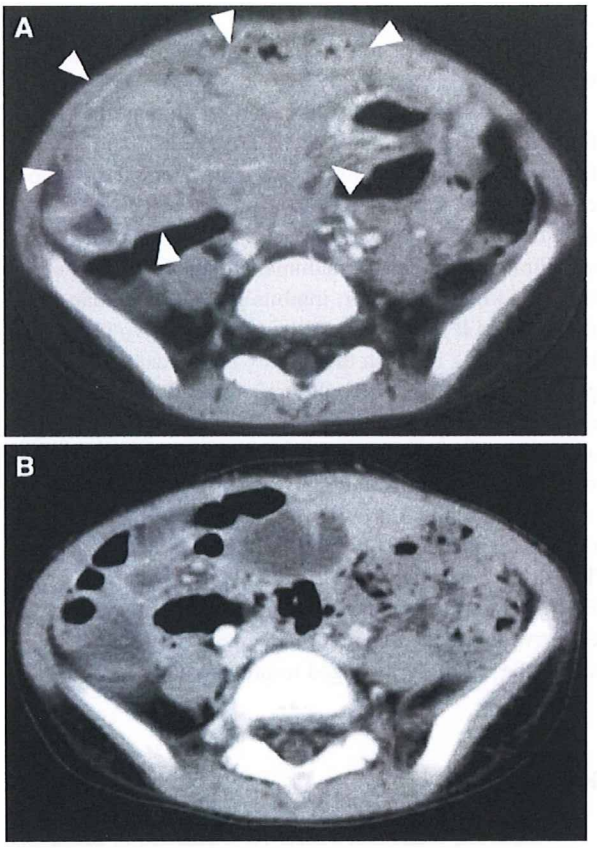

Fig. 2 CT scan images. Abdominal enhanced CT scan showing an ileocecal mass (*arrowheads*) at diagnosis (**a**) and dramatic disappearance after the first course of combination chemotherapy (**b**)

origin and contained EBV in lesions [1, 2]. Polymorphic disease is manifested as reactive B cell hyperplasia or B cell lymphoma, while monomorphic diseases as diffuse large B cell lymphoma or Burkitt lymphoma [2, 4].

Our patient developed monomorphic PTLD, DLBL with *t*(8;14)(q24;q32) possessing *c-Myc* rearrangement, which strongly suggests the existence of immunoglobulin heavy chain (IgH)/*c-Myc* fusion. *t*(8;14)(q24;q32) has been detected not only in patients with Burkitt lymphoma but also in 5–15% of patients with de novo DLBL [6, 7]. This translocation results in overexpression of *c-Myc*, driving cell growth and proliferation, and expression of other genes involved in cell growth [8]. Extranodal lymphomas, particularly gastrointestinal lymphomas, as observed in our case, are more likely to carry *c-Myc* rearrangement than nodal lymphoma [7]. The role of chronic infection with EBV in the pathogenesis of a variety of tumors including Burkitt lymphoma is well documented. However, the mechanisms involved have not been completely defined. EBV might have an initiating role in which growth-transforming B cell infections

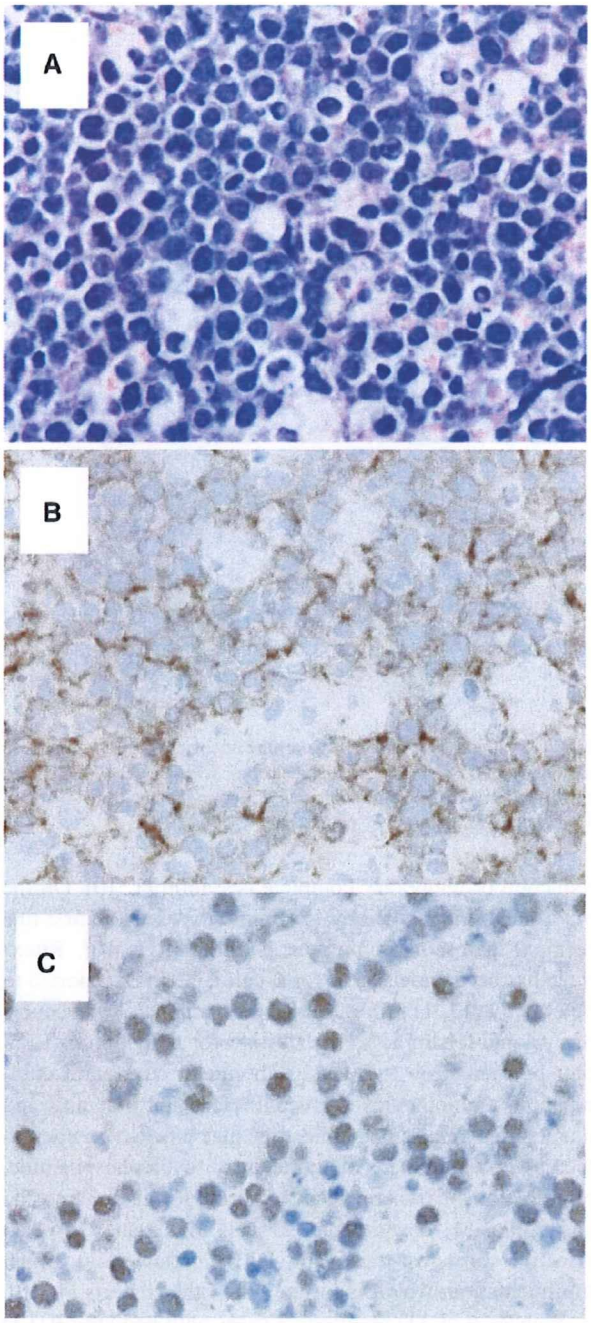

Fig. 3 Pathological studies. **a** Diffuse infiltration of large lymphocytes with clear nucleoli (H&E stain, ×400). **b** Tumor cells showing strong CD20 immunoreactivity stained brown (CD20 stain, ×400). **c** Tumor cells showing reactivity stained brown with the probe specific for EBER-RNA (EBER in situ hybridization, ×400)

establish a pool of target cells that are at risk of a subsequent *c-Myc* translocation [9].

Most patients with polymorphic disease are treated with lowered immunosuppression in most institutes: minor

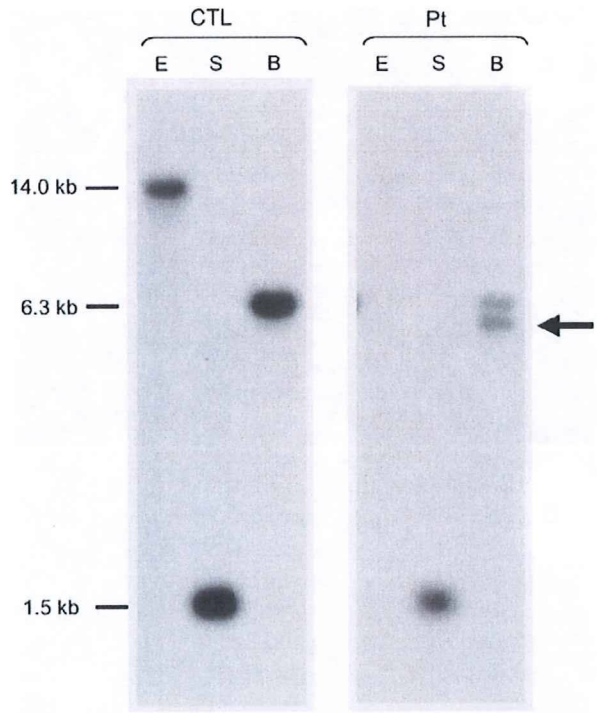

Fig. 4 Southern blot analysis. Rearrangement of the *c-Myc* gene in tumor cells in ascites by Southern blotting with *Eco*RI (E), Sac I(S) and Bgl II (B). The *arrow* indicates a rearrangement band of the *c-Myc* gene. *CTL* control, *Pt* patient

reduction or temporary complete cessation [1, 10]. On the other hand, chemotherapy is commonly used for first-line therapy against monomorphic lymphoma [1, 10]. Rituximab has been widely used for B cell PTLD and reported to be effective [2, 11]. In our case, rituximab and one course of cyclophosphamide were effective for reduction of EBV titer by more than two logs. Subsequent courses of combination chemotherapy were sufficient for inducing and maintaining remission, indicating that block-type chemotherapy containing methotrexate, cyclophosphamide, pirarubicin and dexamethasone, or cytosine arabinoside and etoposide, designed for B cell malignancy, is also effective for post-transplant DLBL after solid organ transplantation, which is in line with other reports [1, 10].

Webber et al. [1] reported 42 cases with pediatric PTLD after cardiac transplantation, of which 16 patients died from progressive PTLD ($n = 7$), acute rejection ($n = 3$), coronary artery disease ($n = 3$), PTLD with acute rejection ($n = 1$), PTLD with sudden death ($n = 1$), and graft failure ($n = 1$). This report also indicated that death from graft loss is a serious issue during PTLD treatment. A fine balance between management against PTLD and preserving allograft from rejection is therefore highly important. With respect to this standpoint, chemotherapy may be useful for maintaining an immunosuppressed state to prevent allograft rejection [1, 10]. Lower rejection rates have been reported when chemotherapy was used as primary therapy [1].

In our case, CyA was continued without further reduction during the treatment of PTLD to protect the allograft from rejection. Following cessation of chemotherapy, oral everolimus was initiated with a further reduction of CyA dose to maintain trough levels at 30–60 ng/mL. Everolimus, an immunosuppressive mammalian target of rapamycin (mTOR) kinase inhibitor, inhibits growth of human EBV-transformed B lymphocytes in vitro and in vivo [12]. Thus, it is promising agent in that it may be effective in both the prevention of PTLD and allograft rejection. In our case it might have contributed to protection against PTLD relapse as well as allograft rejection. Further studies are needed with this agent in this clinical setting.

In summary, we present a child who was successfully treated for post-transplant DLBL after cardiac transplant. The disease was associated with *c-Myc* rearrangement and EBV. Rituximab and combination chemotherapy were effective in inducing and maintaining remission. PTLD should be carefully managed to prevent allograft rejection.

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LETTER TO THE EDITOR

Two rare MPL gene mutations in patients with essential thrombocythemia

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In contrast to polycythemia vera, approximately half of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) are negative for JAK2-V617F [1]. Two missense mutations in Codon 515 of the thrombopoietin receptor (TpoR/MPL) gene (W515L and W515K) have been found in a small portion (1–9%) of ET and PMF patients [2–4], and several other MPL exon 10 mutations have also been reported [5]. In the process of examining samples of 20 JAK2-V617F-negative ET patients for MPL mutations, other than three patients with W515K, we found two patients with sequence alterations that had not been reported in ET patients.

UPN-1 and UPN-2 are women and did not have family history of hematological disorders. They were referred for thrombocytosis at the age of 45 years in 2006, and 33 years in 2003, respectively. Platelet counts at diagnosis were 1,884,000/mm^3 for UPN-1, and 951,000/mm^3 for UPN-2. After diagnosis, they both were treated with an antiplatelet agent but not with a cytoreductive agent. They had neither thrombotic events nor leukemic transformation. Clinical characteristics of these patients, both at diagnosis and at examination, met the WHO criteria of ET (2008).

Direct sequencing (DS) of granulocytes (GCs) in UPN-1 for exon 10 of the MPL gene revealed overlapping peaks at 515 and subsequent codons, although for T-lymphocytes (TLs), no such peaks were detected (Fig. 1a, upper panel). Sequencing of cloned PCR products revealed deletion of 10 nucleic acids and insertion of four unrelated sequences (Fig. 1a, lower panel). Figure 1b shows nucleic acid and resulting amino acid changes found in UPN-1. Four amino acids encoded by codons 515 to 518, namely Tryptophan, Glutamine, Phenylalanine, and Proline, were lost, and two amino acids, Lysine and Threonine, were inserted. We designated this mutation as W515-P518delinsKT. To the best of our knowledge, this mutation has not been described in any clinical samples. Codon 515, which encodes Tryptophan and the hot spot of MPL mutation, as well as five amino acid residues encoded by codons 514 to 518 (Fig. 1b), known as the amphipathic motif, are important for the prevention of autonomous activation of TpoR [6]. Thus, although functional analysis of this mutation has not been performed, it is likely that W515-P518delinsKT was the causative mutation of ET in this patient.

DS of GCs in UPN-2 revealed overlapping sequences at Codon 515 of the MPL gene (Fig. 1c). The same additional peaks were detected in TLs, but with much lower height. Sequencing of cloned PCR product confirmed conversion of two nucleic acids (TGG > GCG) in Codon 515 (data not shown), which would result in an amino acid change from Tryptophan to Alanine. This mutation, designated W515A, had been reported in a patient with PMF [5]. The transforming potential of this mutation was confirmed in functional analysis of Tpo-R [6].

Absence of a mutant sequence or very low mutant allele ratio in TLs confirmed that both W515-P518delinsKT and W515A are somatically acquired mutations (Fig. 1a, c). This is an important issue for MPL mutations, as the same MPL mutation (S505N) has been reported as both acquired (sporadic ET case) and as germline (pedigree of familial ET) [4, 7], and it was not established in the PMF case with

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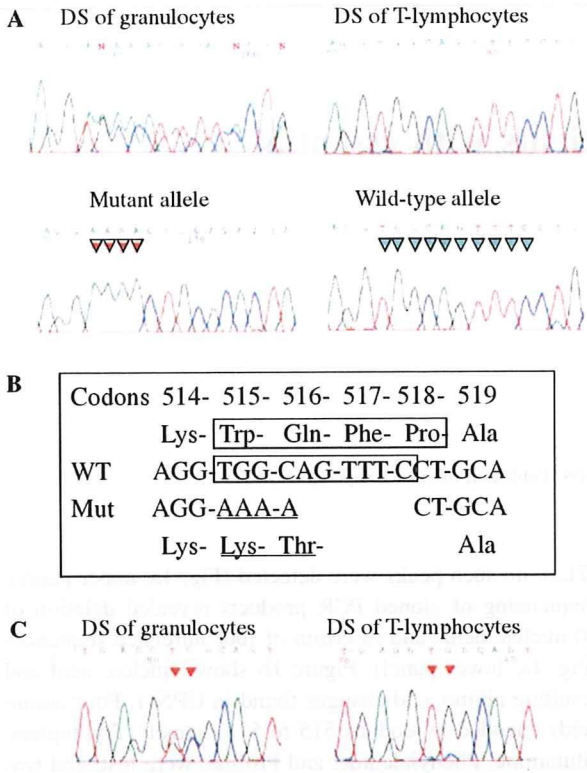

Fig. 1 Mutation analysis of the MPL gene in UPN-1 and UPN-2. **a** The results in UPN-1. DS in GCs and TLs (*upper pannel*), and sequencing of cloned PCR products (mutant and wild-type alleles, *lower pannel*) are shown. *Arrowheads* indicate inserted (*left*) and deleted (*right*) sequences. **b** Nucleotide and resulting amino acid changes in the MPL gene of UPN-1. *Boxed* sequences (amino acids and nucleotides) are deleted, and *underlined* sequences are inserted. **c** The results in UPN-2. The results of DS in GCs and TLs are shown. *Arrowheads* indicate mutated sequences

W515A due to a lack of control sample [5]. The fact that two of the five MPL exon 10 mutations that we found among 20 JAK2-V617F-negative ET patients were mutations other than W515L or W515K may suggest the possibility that allele-specific PCR for these common mutations may not be sufficient for screening of MPL mutations in ET and PMF.

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ORIGINAL ARTICLE

Outcome of allogeneic bone marrow transplantation from unrelated donors for adult Philadelphia chromosome-negative acute lymphocytic leukemia in first complete-remission

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Abstract The indication of allogeneic stem cell transplantation (allo-SCT) for Philadelphia chromosome-negative acute lymphocytic leukemia [Ph(−) ALL] from unrelated donors is not established. To assess its potency of unrelated patients in first complete-remission (CR1) transplanted from unrelated donors and the potential prognostic factors affecting the probability of survival, we retrospectively analyzed a total of 41 adult Ph(−) ALL patients in CR1 who underwent unrelated bone marrow transplantation at 6 transplantation centers of the Nagoya Blood and Marrow Transplantation Group between 1993 and 2006. The median age of the 41 patients was 28 years (range, 18–51 years). HLA was matched in 33 transplants, with mismatches in 8 (HLA-A allele mismatch:1, HLA-DR serological mismatch: 2, HLA-DRB1 mismatch: 5). Leukemia-free survival (LFS) at 3 and 6 years from allo-SCT was 60.3 and 47.7%, respectively. LFS at 5 years was 62.1% for those transplanted from HLA-matched donors. LFS was significantly lower with HLA-mismatched donors due to higher transplantation-related mortality. Relapse was observed in 3 patients. Our study suggested that unrelated allo-SCT could improve LFS of patients with a potential graft-versus-leukemia effect. Unrelated allo-SCT for Ph(−) ALL patients in CR1 could be more beneficial by reducing TRM, such as selecting a HLA-matched donor.

Keywords Acute lymphocytic leukemia · Allogeneic stem cell transplantation from unrelated donors · First complete-remission

For the Nagoya Blood and Marrow Transplantation Group.

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1 Introduction

The prognosis of adult acute lymphocytic leukemia (ALL) is still unsatisfactory. Although several adverse prognostic factors, such as higher age, high WBC counts at diagnosis, cytogenetics including t(9;22), late achievement of complete-remission (CR), were proposed in terms of chemotherapy for adult ALL [1–3], it is unclear that these factors could always be applied as risk factors for patients who received allogeneic stem cell transplantation (allo-SCT). Whereas the rate of CR in adult ALL is considerably high (78–93% in major clinical trials), long-term leukemia-free survival (LFS) achieved in only around 30–40% of patients, suggesting that there is still plenty of room to improve post-consolidation therapy [1, 2, 4–9].

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Allo-SCT represents a most potent post-consolidation therapy and a curative option for ALL. The outcome of allo-SCT after first relapse was very poor in the past studies [10, 11], therefore disease status at allo-SCT for ALL is an important factor, and better results for adult ALL patients can be obtained by performing allo-SCT in first complete-remission (CR1). For high-risk patients with poor prognostic chromosomal abnormalities, defined by the presence of t(9;22), t(4;11), or t(1;19), allo-SCT in CR1 is the treatment of choice. However, the indication of allo-SCT for adult standard-risk ALL in CR1 is still controversial. Although it was reported that sibling allo-SCT for ALL in CR1 could be recommended for standard-risk patients [12, 13], unrelated allo-SCT for standard-risk ALL in CR1 remains controversial [14]. Another report demonstrated no difference between related compared with unrelated allo-SCTs in adult ALL patients [15]. To assess its potency of unrelated allo-SCT, we retrospectively analyzed the outcome of adult Philadelphia chromosome-negative [Ph(−)] ALL CR1 patients transplanted from unrelated donors and the potential prognostic factors affecting the probability of survival.

2 Patients and methods

The study population consisted of 41 patients with adult Ph(−) ALL who received unrelated bone marrow transplantation between September 1993 and October 2006 at 6 transplant centers of the Nagoya Blood and Marrow Transplantation Group (NBMTG). Study admission criteria were adults, receiving allo-SCT for the first time, from unrelated donors, with non-manipulated graft and with Ph(−) ALL. Diagnosis was based on cytology, karyotype, and immunophenotyping of leukemia cells. There was no Burkitt leukemia (ALL-L3), typically defined as t(8;14) in WHO classification. Patients received induction and consolidation chemotherapy according to local protocols and proceeded to allo-SCT according to indications by the individual transplant centers. This is a multi center study for longer than one decade and therefore chemotherapies were not uniform and detailed regimens were described previously (Japan Leukemia Study Group (JALSG) ALL-87 [16], ALL-90 [17], ALL-93 [2], ALL-97, ALL-202, or hyper-CVAD [18]). Briefly, induction therapies contained 5 drug-containing regimens (vincristine, prednisolone, L-asparaginase, cyclophosphamide and doxorubicin or daunomycin) followed by intensive consolidation chemotherapies. Drugs commonly used for maintenance chemotherapy were methotrexate, 6-mercaptopurine, vincristine, cyclophosphamide and prednisolone. Protocols were approved by local institutional review boards, and all patients provided informed consent.

2.1 Donors and transplant procedures

Donors were selected based on a combination of serotyping and genotyping performed for HLA-A, -B and -DRB1 according to standard procedures. Donor and recipient pairs were considered matched when identical at HLA-A, -B, and -DRB1 loci. Mismatches included at least one disparity at one of these loci. As conditioning regimens, cyclophosphamide-based conditioning regimens included cytarabine 8 g/m^2 + cyclophosphamide 120 mg/kg + total body irradiation (TBI) 10–13.2 Gy and cyclophosphamide 120 mg/kg + TBI 10–13.2 Gy, melphalan-based conditioning regimens were melphalan 150–180 mg/m^2 + TBI 8–12 Gy, and non-TBI regimens included busulfan 16 mg/kg + cyclophosphamide 120 mg/kg and melphalan 140–180 mg/kg + fludarabine 125 mg/m^2. We used cyclosporine A plus short-term methotrexate (CyA + sMTX) or tacrolimus plus short-term methotrexate (Tac + sMTX) for graft-versus-host disease (GVHD) prophylaxis, according to the choice of each transplant center. Supportive care measures were taken in accordance with local protocols.

2.2 End points and statistical analysis

End points of this study included engraftment, GVHD, transplantation-related mortality (TRM), relapse, overall survival (OS) and LFS. Neutrophil engraftment was defined as the first of 3 consecutive days when the absolute neutrophil count exceeded 0.5×10^9/L, and platelet engraftment was defined as the first of 3 consecutive days when the absolute platelet count exceeded 20×10^9/L without platelet transfusion. Acute and chronic GVHD were diagnosed and graded according to consensus criteria [19, 20]. Chronic GVHD was evaluated among patients who survived at least 100 days after allo-SCT. Definitions of OS, LFS, TRM and relapse were according to standard criteria [21]. Event-related data were measured from the date of allo-SCT to that of disease relapse or death from any cause. A Chi-square test was used to evaluate the correlation between two groups, and a Mann–Whitney's *U* test for a group comparison. Kaplan–Meier product-limit estimates were performed to determine OS and LFS, while the different subgroups were compared for significance using the log-rank test [22, 23]. The cumulative incidence function was used for TRM [24, 25]. The Cox proportional hazards model was used for univariate analyses to determine risk factors [26]. As parameters, we analyzed HLA disparity (match vs. mismatch), age at allo-SCT (>40 vs. ≤40 years old), WBC count at diagnosis ($\geq 30 \times 10^9$ vs. $<30 \times 10^9$/L), lineage (B cell vs. T cell vs. B + T), cytogenetics [normal vs. t(4;11) vs. others], treatment response (CR after one course of induction vs. CR after more than one course of induction), conditioning regimen

[cyclophosphamide-based regimens + TBI vs. melphalan-based regimens + TBI vs. non-TBI regimens], GVHD prophylaxis (CyA + sMTX vs. Tac + sMTX). A significance level of $P < 0.05$ was used for all analysis. The analyses were based on all data available as of November 2006.

3 Results

3.1 Patient characteristics

We retrospectively examined 41 adult Ph(−) ALL patients (male: 21, female: 20). Median age at allo-SCT was 28 years (range, 18–51). All patients received bone marrow transplantation through the Japan Marrow Donor Program (JMDP), because currently JMDP provides bone marrow graft only. HLA was matched in 33 patients, with mismatches in 8 (HLA-A allele mismatch: 1, HLA-DR serological mismatch: 2, HLA-DRB1 mismatch: 5). HLA-mismatch transplantation may be considered because in the Japanese population lower incidence of GVHD and feasible outcome of HLA-DR or -DRB1 mismatched transplantation were reported in the past reports [27, 28]. Conditioning regimens were cyclophosphamide-based conditioning regimens in 26 patients, melphalan-based conditioning regimens in 10 and non-TBI regimens in 5. For GVHD prophylaxis, CyA + sMTX were used in 19 patients, Tac + sMTX in 22 (Table 1).

3.2 Engraftment

Neutrophil engraftment was achieved in all patients at a median 16 (range, 12-21) days post-transplant. All the available data from 18 patients who received a sex-mismatched graft showed more than 90% donor type within 100 days after allo-SCT using XY fluorescence in situ hybridization of bone marrow. In 4 patients, data using short tandem repeats/variable number tandem repeats were available, and all patients achieved more than 90% donor chimerism within 100 days after allo-SCT.

3.3 Acute and chronic GVHD

The overall incidence of acute GVHD (aGVHD) was 68.3% (grade II–IV: 48.4%, and grade III–IV: 12.2%, respectively). Whereas LFS in patients with grade I–II aGVHD (56.9% at 5 years) was significantly superior to that in those with grade III–IV aGVHD (20.0% at 5 years) ($P = 0.04$), LFS in patients with grade I–II aGVHD was not significantly different from that in patients without aGVHD (68.8% at 5 years) ($P = 0.65$). Cumulative incidence of grade II–IV acute GVHD at day 100 was 59.2% for those transplanted from HLA-matched donors, and 48.6% for those transplanted from HLA-mismatched donors ($P = 0.41$).

Among 37 patients who survived at least 100 days after allo-SCT, the overall incidence of chronic GVHD (cGVHD) was 44.4% (limited type: 8.1%, extensive type: 35.1%, respectively). Three of 37 patients with cGVHD (8.1%) were of the de novo type. Other patients had cGVHD following aGVHD (quiescent onset or progressive onset). The presence or absence of cGVHD did not affect LFS (patients with cGVHD: 58.7% vs. patients without cGVHD: 66.0% at 5 years; $P = 0.95$). Cumulative incidence of chronic GVHD at 2 years was 45.2% for those transplanted from HLA-matched donors, and 20.0% for those transplanted from HLA-mismatched donors ($P = 0.30$).

3.4 Survival

After a median follow-up of 29.4 months (range, 1.3–146.4 months), 22 of 41 patients (53.7%) were alive. OS rates at 2 years were 74.4% for those transplanted from HLA-matched donors, and 31.3% for those transplanted from HLA-mismatched donors ($P = 0.02$).

LFS rates at 3 and 6 years were 60.3 and 47.7%, respectively. In univariate analyses, HLA-mismatch was identified as the only negative prognostic parameter for LFS, other factors did not show significant influence (Table 2). LFS rates were significantly higher in patients transplanted from HLA-matched donors compared with those from HLA-mismatched donors (71.5 vs. 31.3% at 2 years; $P = 0.03$) (Fig. 1).

3.5 Causes of death

Transplantation-related mortality occurred in 16 out of 41 patients (39.0%) at a median 217 (range, 41–2436) days post-transplant. The cumulative incidence of TRM was 40.4% at 5 years from allo-SCT. Within 5 years after allo-SCT, 13 patients died of TRM due to: infection ($n = 5$), non-infectious pulmonary dysfunction (NIPD) ($n = 5$) and GVHD ($n = 3$). Three patients died more than 5 years after allo-SCT caused by secondary malignancy ($n = 2$), NIPD ($n = 1$) (Table 3). Univariate analyses identified HLA-mismatch as the only risk factor [HLA-mismatch: HR 3.56 (95% CI 1.18–10.8); $P = 0.02$]. There were six NIPD cases consisting of 4 interstitial pneumonitis, 1 bronchiolitis obliterans, and 1 idiopathic pneumonia syndrome. A Chi-square test showed no correlation between NIPD and both acute GVHD ($P = 0.21$) and chronic GVHD ($P > 0.99$). The incidence of NIPD was higher in patients transplanted from HLA-mismatched donors; 3 out of 8 (37.5%) in patients transplanted from HLA-mismatched donors and 3 out of 33 (9%) in those from HLA-matched

Table 1 Patient characteristics

Characteristics of patients	No. of evaluable	HLA-matched	HLA-mismatched	*P* value
No. of patients		33	8	
Age, median (years) (range)	41	28 (18–51)	35.5 (23–46)	0.21
<30		19	3	0.31
≥30		14	5	
Sex	41			0.48
Male		16	5	
Female		17	3	
WBC at diagnosis ($\times 10^9$/L), median (range)	40	15.4 (1.4–480)	11.5 (1.4–55.5)	0.42
$<30 \times 10^9$/L		22	6	0.73
$\geq 30 \times 10^9$/L		10	2	
Lineage	38			0.77
B		18	3	
B + T		2	1	
T		9	3	
Null		2	0	
Cytogenetics	35			0.40
Normal		22	3	
t(4;11)		1	0	
Others (not include hyperdiploid)		6	3	
Chemotherapy regimen	40			0.31
ALL-93		10	1	
ALL-97		10	2	
ALL-202		3	3	
Hyper-CVAD		2	0	
Others		7	2	
Induction courses to CR1	41			0.09
1		28	5	
2		5	2	
3		0	1	
Time from diagnosis to transplantation (months)	41	10.1 (4.8–22.3)	10.7 (7.4–14.7)	0.67
Conditioning regimen	41			0.38
CY + TBI with or without others		21	5	
L-PAM + TBI		9	1	
Non-TBI regimens		3	2	
Donor age, median (years) (range)	35	33 (20–49)	33.5 (25–47)	0.89
Donor-recipient sex match	39			0.90
M–M		11	4	
M–F		9	2	
F–M		5	1	
F–F		6	1	
GVHD prophylaxis	41			0.18
CyA + sMTX		17	2	
Tac + sMTX		16	6	
Median (range) follow-up of survivors (months)		37.2 (2.5–146.4)	32.7 (4.9–35.9)	0.27

CR1 first complete-remission, *CY* cyclophosphamide, *TBI* total body irradiation, *L-PAM* melphalan, *GVHD* graft-versus-host disease, *CyA* cyclosprine, *sMTX* short-term methotrexate, *Tac* tacrolimus

Table 2 Univariate analyses of factors influencing LFS

Risk factor	HR (95% CI)	*P* value
HLA-mismatch	3.24 (1.06–9.26)	0.04
Age > 40 years	1.21 (0.40–3.68)	0.74
WBC ≥ 30 × 10^9/L	1.12 (0.43–2.96)	0.82
Liniage (vs. B)		
B + T	1.01 (0.21–4.69)	0.99
T	0.44 (0.05–3.73)	0.45
Induction more than one course	1.78 (0.58–5.48)	0.31
Conditioning (vs. non-TBI)		
CY + TBI with or without others	0.71 (0.20–2.54)	0.59
L-PAM + TBI	0.81 (0.19–3.43)	0.78
GVHD prophylaxis (vs. CyA + sMTX)		
Tac + sMTX	0.93 (0.36–2.42)	0.88

CY cyclophosphamide, *TBI* total body irradiation, *L-PAM* melphalan, *GVHD* graft-versus-host disease, *CyA* cyclosporine, *sMTX* short-term methotrexate, *Tac* tacrolimus

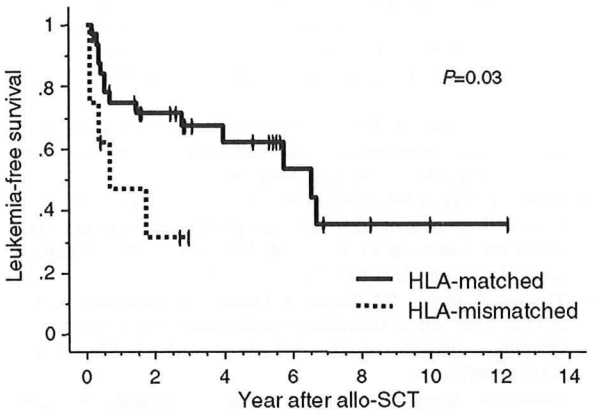

Fig. 1 Leukemia-free survival (LFS) according to HLA disparity. LFS at 2 years was 71.5% for those transplanted from HLA-matched donors, and 31.3% for those transplanted from HLA-mismatched donors ($P = 0.03$). LFS at 5 years was 62.1% for those transplanted from HLA-matched donors

donors. A Chi-square test showed a weak association between HLA-mismatch and NIPD ($P = 0.06$). Relapse was observed in only 3 of 41 patients (7.3%) at a median 524 (range, 132–624) days post-transplant, and all of them died because of leukemia.

4 Discussion

In this study, we demonstrated the outcome of unrelated allo-SCT in adult Ph(−) ALL in CR1. LFS of those who transplanted from HLA-matched donors was relatively favorable (62.1% at 5 years). In the UK Medical Research Council UKALL XII/Eastern Cooperative Oncology Group E2993 trial, OS rates at 5 years were reported 53% in sibling allo-SCT [12]. On the other hand, in the Center for International Blood and Marrow Transplant Research, the OS rates at 5 years were 39% in unrelated allo-SCT [14]. Our result of unrelated bone marrow transplantation was close to that reported in sibling allo-SCT from western countries. This is probably associated with the low incidence of acute and chronic GVHD in the Japanese population, which is thought to be the result of genetic homogeneity [27, 29, 30]. Considering the fact that most newly diagnosed patients once obtain CR, unrelated allo-SCT in CR1 could be a realistic option to achieve a cure.

Table 3 Causes of death

	HLA-matched	HLA-mismatched
No. of deaths	14	5
Relapse	2	1
Infection	5	0
GVHD	2	1
NIPD	3	3
Secondary malignancy	2	0

GVHD graft-versus-host disease, *NIPD* non-infectious pulmonary dysfunction

While relapse would be the main problem in Ph(−) ALL patients treated with chemotherapy, relapse rate was considerably low in CR1 patients transplanted from unrelated donors in our study. By analyzing the causes of death, TRM accounted for most of them, including relatively high tendency of NIPD which might be associated with HLA-mismatch. Thus reducing TRM could improve the results of allo-SCT for standard-risk Ph(−) ALL. In fact, it was reported that a lengthy interval from diagnosis to transplantation adversely affected the incidence of interstitial pneumonitis [31, 32], bronchiolitis obliterans [33], and idiopathic pneumonia syndrome [34]. Some authors suggested that this reflected the cumulative toxic sequelae of chemotherapy administered prior to allo-SCT [31, 35]. Many of the drugs commonly used for ALL, such as cyclophosphamide and methotrexate, are known to cause interstitial lung disease [36]. It might be able to reduce TRM by considering timing for performing allo-SCT and reducing cumulative toxicity of chemotherapy as well as avoiding HLA-mismatched allo-SCT.

The incidence of second malignancies after chemotherapy was reported 8–12% at 20 years [37], and leukemia is the most frequent secondary malignancy [38]. Although the data for secondary malignancies after chemotherapy for adult ALL was limited, the cumulative risk of secondary leukemia was reported 3.63% at 10 years in a past report [39]. On the other hand, it was reported that

second malignancies after allo-SCT accounted for 6% of the late deaths [32], and the incidence of solid malignancies was reported 4.2% at 10 years after allo-SCT in NBMTG [40]. In this study, secondary malignancies were observed in 2 patients out of 41 (4.9%), being compatible with the past reports. Although the incidence of solid malignancies would be greater for patients performed allo-SCT compared with those performed chemotherapy for adult ALL, it may not be necessarily the case that second malignancies would be observed more frequently in allo-SCT.

Known risk factors in administering chemotherapy, such as WBC count at diagnosis, chromosomal abnormalities and response to induction therapy [3, 41, 42] did not affect LFS, suggesting that allo-SCT could overcome the known risk factors for chemotherapy. For Japanese population, Takeuchi et al. [2] reported that the overall survival of 263 adult ALL patients who received chemotherapy in the JALSG ALL-93 study was 23% at 6 years, and that the OS rate for the low-risk group was 53%, against 33 and 15% for the intermediate-risk group and high-risk group, respectively. Although it is incommensurable, our data suggested a possible beneficial effect of unrelated allo-SCT from a suitable donor. Further study to compare allo-SCT with chemotherapy in patients who has no suitable sibling donor is warranted to determine the possible role of unrelated allo-SCT in CR1 ALL.

In this study, data were obtained retrospectively from allo-SCT spanning longer than one decade with heterogeneity in induction and transplant regimens. Although LFS was not significantly different between patients transplanted before 1999 and those after 2000 (data not shown), the development of new drugs and transplant procedures including non-myeloablative conditioning and expanded indication for elderly patients, and duration of follow-up may have a potential bias associated with the results. And there may also be a potential selection bias performing allo-SCT for patients with good conditions. This is the limitation of retrospective studies where the indication of allo-SCT was based on each transplant center.

In conclusion, our data suggested that LFS of adult Ph(−) ALL CR1 patients transplanted from unrelated donors could be improved by reducing TRM. Especially those with known risk factors might be able to achieve better LFS. To confirm the indication of unrelated allo-SCT in adult Ph(−) ALL, further retrospective studies to confirm the current results with larger patient cohorts are warranted.

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