Conditioning regimen	Myeloablative	243 (74%)	338 (75%)	0.883
	Reduced-intensity	84 (26%)	114 (25%)	
GVHD prophylaxis	Cyclosporine-based	113 (35%)	108 (24%)	0.004
	Tacrolimus-based	209 (64%)	338 (75%)	
	Others	5 (2%)	6 (1%)	
Use of	Yes	33 (10%)	13 (3%)	<0.001
ATG/alemtuzumab	No	294 (90%)	439 (97%)	
Follow-up of survivors	Median time† (range)	36.2 (3.0-95.7)	13.5 (1.7-62.8)	<0.001

Data are numbers (%) unless specified otherwise.

Abbreviations: AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; HVG, host-versus-graft; GVH, graft-versus-host; GVHD, graft-versus-host disease; cyclosporine-based, cyclosporine with or without other agents; tacrolimus-based, tacrolimus with or without other agents; ATG, antithymocyte globulin; RD/1AG-MM-GVH, related donor with 1-antigen mismatch in the GVH direction; 8/8 MUD, HLA-8/8-allele-matched unrelated donor.

*HLA compatibility was defined according to HLA-A, -B, and -DR loci.

†Data are expressed in months.

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Table 2. Multivariate analysis of overall survival

Variable	Total (n = 779)		Standard risk (n = 492)		High risk (n = 262)	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Donor type						
8/8 MUD	1.00	-	1.00	-	1.00	-
RD/1AG-MM-GVH	1.49 (1.19-1.86)	<0.001	1.72 (1.24-2.39)	0.001	1.30 (0.96-1.76)	0.095
Age						
<=50	1.00	-	1.00	-		
>50	1.44 (1.16-1.79)	0.001	1.55 (1.13-2.15)	0.007		
Performance status						
0/1	1.00	-			1.00	-
2/3/4	1.79 (1.30-2.48)	<0.001			1.76 (1.24-2.52)	0.002
Disease risk						
Standard	1.00	-				
High	2.41 (1.92-3.03)	<0.001				
Unknown	1.38 (0.82-2.33)	0.227				

Only variables that remained after backward selection in the multivariate analysis are shown.

Abbreviations: 8/8 MUD, HLA-8/8-allele-matched unrelated donor; RD/1AG-MM-GVH, related donor with 1-antigen mismatch in the graft-versus-host direction; CI, confidence interval.

Table 3. Multivariate analysis of overall survival in patients receiving transplantation from a related donor with a 1-antigen mismatch in the GVH direction

Variable	Total (n = 327)		Standard risk (n = 175)		High risk (n = 133)	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
HLA mismatch in the GVH direction						
HLA-DR mismatch	1.00	-	1.00	-	1.00	-
HLA-A mismatch	1.07 (0.73-1.56)	0.737	0.98 (0.54-1.81)	0.966	1.11 (0.65-1.89)	0.701
HLA-B mismatch	1.57 (1.13-2.18)	0.007	1.86 (1.14-3.01)	0.012	1.36 (0.86-2.17)	0.193
HLA mismatch in the HVG direction						
0-1 mismatches	1.00	-	1.00	-	1.00	-
2-3 mismatches	1.27 (0.91-1.76)	0.154	1.67 (0.98-2.85)	0.061	1.06 (0.69-1.61)	0.799
Age						
<=50	1.00	-	1.00	-		
>50	1.52 (1.14-2.03)	0.004	1.87 (1.21-2.91)	0.005		
Disease risk						
Standard	1.00	-				
High	2.06 (1.53-2.78)	<0.001				
Unknown	1.00 (0.53-1.89)	0.989				
Source of stem cells						
Bone marrow						
Peripheral blood						

Only variables that remained after backward selection in the multivariate analysis are shown.

Abbreviations: GVH, graft-versus-host; HVG, host-versus-graft; CI, confidence interval.

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Table 4. Multivariate analysis of treatment-related mortality and relapse in patients with standard-risk diseases receiving transplantations from a related donor with a 1-antigen mismatch in the GVH direction

Variable	Treatment-related mortality (n = 164)		Relapse (n = 164)	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
HLA mismatch in the GVH direction				
HLA-DR mismatch	1.00	-	1.00	-
HLA-A mismatch	1.22 (0.59-2.52)	0.587	0.70 (0.29-1.67)	0.418
HLA-B mismatch	2.00 (1.09-3.65)	0.025	0.80 (0.34-1.87)	0.605
HLA mismatch in the HVG direction				
0-1 mismatches	1.00	-	1.00	-
2-3 mismatches	2.21 (1.14-4.28)	0.019	0.67 (0.23-1.98)	0.467
Age				
<=50	1.00	-		
>50	2.08 (1.18-3.65)	0.011		
Duration from diagnosis to transplant				
<6 months	1.00	-		
>=6 months	2.40 (1.19-4.82)	0.014		
Unknown	2.23 (0.77-6.48)	0.140		

Only variables that remained after backward selection in the multivariate analysis are shown.

Abbreviations: GVH, graft-versus-host; HVG, host-versus-graft; CI, confidence interval.

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Figure legends

Figure 1. Overall survival according to donor type and risk of disease

Overall survival after transplantation from a related donor with an HLA-1-antigen mismatch in the graft-versus-host direction (RD/1AG-MM-GVH), HLA 8/8-allele-matched unrelated donor (8/8 MUD), and HLA-matched related donor (MRD) in patients with both-risk (A), standard-risk (B), or high-risk diseases (C). Survival rates in the 8/8 MUD and RD/1AG-MM-GVH groups were compared by the log-rank test.

Figure 2. Overall survival in patients with both-risk (A, B), standard-risk (C, D) or high-risk diseases (E, F) according to the locus of HLA mismatch in the GVH direction, and the number of mismatches in the HVG direction

Survival rates in patients with HLA-A, -B, and -DR mismatch in the GVH direction were compared by the log-rank test (A, C, E). Survival rates in patients with HLA 0-1 mismatch and 2-3 mismatches in the HVG direction were compared by the log-rank test (B, D, F). Survival rate of 8/8 MUD group are shown for visual comparison. 8/8 MUD, HLA 8/8-allele-matched unrelated donor.

Figure 3. Cumulative incidence of treatment-related mortality (A) and relapse (B) according to the locus of HLA mismatch in the GVH direction in patients with standard-risk diseases

Cumulative incidences in groups of related transplantation were compared by Gray's test. 8/8 MUD, HLA 8/8-allele-matched unrelated donor.

Figure 1.

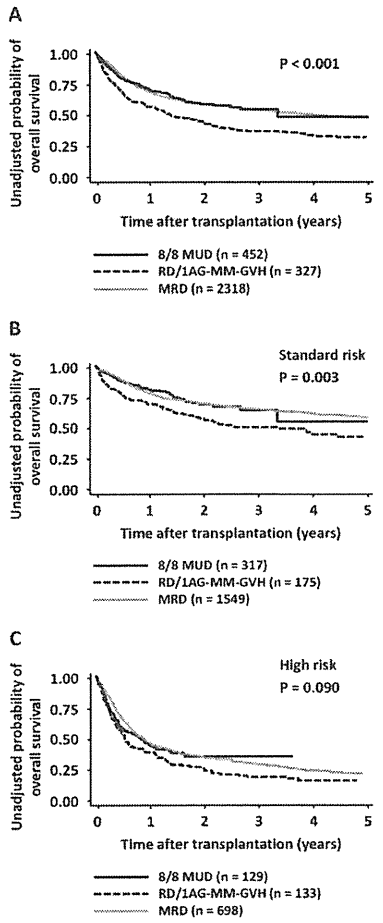

Figure 2.

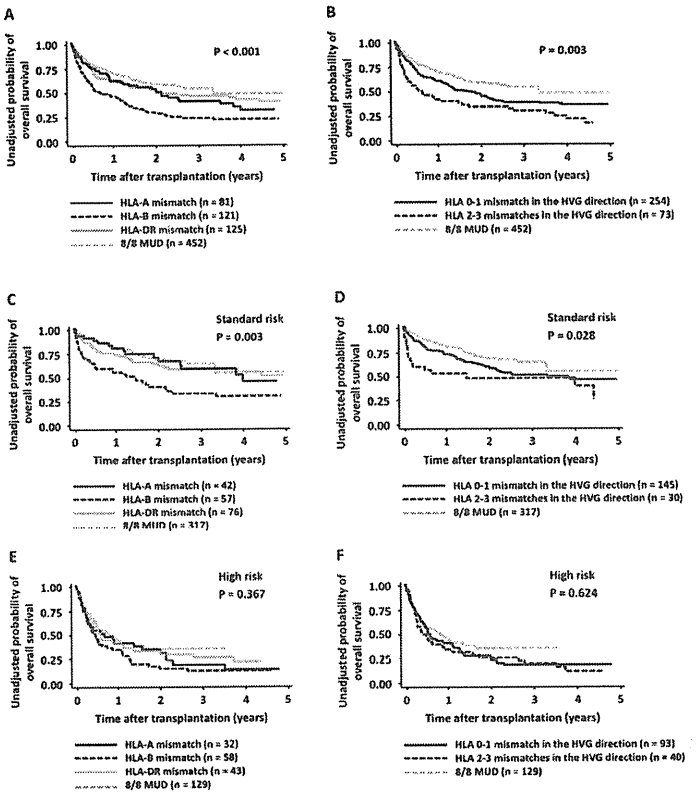

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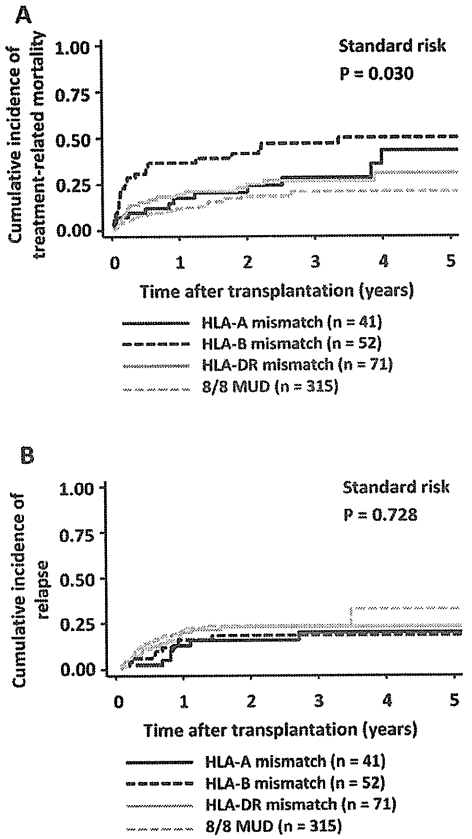
Figure 3.



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Recurrence of monoclonal gammopathy associated with donor-derived myelodysplastic syndrome after cord blood stem cell transplantation

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Myelodysplastic syndrome (MDS) is known to be associated with functional abnormalities of B cells, including hypergammaglobulinemia and monoclonal gammopathy (MG). However, the pathogenesis of these immunological disorders has not been clarified. We report a patient who developed donor-derived MDS followed by leukemic transformation after cord blood transplantation for MDS with MG. Interestingly, MG reappeared before development of donor-derived MDS. We analyzed the immunoglobulin allotype gene polymorphisms to determine whether the MG after cord blood transplantation was of recipient origin or donor origin. Results of genetic analysis and enzyme-linked immunosorbent assay of IgG1 allotype revealed that the MG after cord blood transplantation was of donor origin. Although the mechanism of donor-derived MG remains unclear, the persistent presence of recipient's antigen presenting cells might have induced the abnormal immunoglobulin production.

Myelodysplastic syndromes (MDS), a very heterogeneous group of myeloid malignancies, are associated with significant immunologic abnormalities. Hyper- and hypogammaglobulinemia, monoclonal gammopathy (MG), and autoantibody production are frequently observed in MDS patients as a result of functional abnormalities of B cells [1,2]. In addition, decreased natural killer cell activity, impaired mitogenic response, and diminished CD4-positive cell count have been reported [3,4]. These fundamental immune defects may increase susceptibility to infection, coexisting autoimmune disease, or lymphoid neoplasms. However, the pathogenesis of these immunological disorders has not been clarified.

We experienced a patient who developed donor-derived MDS after cord blood transplantation (CBT) for MDS with MG. Recurrence of MG was observed after CBT and, therefore, we performed immunoglobulin allotype gene polymorphisms analyses to identify the origin of MG after CBT.

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Materials and methods

Case presentation

A 58-year-old Japanese woman with dizziness and general fatigue was diagnosed to have MDS of refractory anemia with excess blasts−2, according to World Health Organization classification in April 2008. The bone marrow blast count was 17% and cytogenetic analysis showed an abnormal chromosome, namely, 47, XX, +8, inv(9)(p12q13), in all dividing cells. She also had a monoclonal increase in IgG κ-chain in the serum and urine without any evidence of myeloma (IgG level, 5480 mg/dL; IgA, 436 mg/dL; IgM, 190 mg/dL; Fig. 1A). Allogeneic bone marrow transplantation (BMT) from a human leukocyte antigen−DR one-antigen−mismatched unrelated female donor was performed in March 2009 by following a nonmyeloablative conditioning regimen of fludarabine at 25 mg/m^2/day on days −7 to −3 and melphalan at 140 mg/m^2/day on day −2. Cyclosporine and short-course methotrexate were given as prophylactic measures for graft-vs-host disease (GVHD). Neutrophil engraftment was observed on day 29 after BMT, however, subsequently, her blood cell count decreased gradually. Bone marrow chimerism analysis on day 48 revealed secondary graft failure. Therefore, CBT from a male donor was performed on day 58 after BMT after administering a conditioning regimen with fludarabine at 30 mg/m^2/day on days −5 to −2 and cyclophosphamide at 30 mg/kg on days −3 and −2. The clinical course early after CBT was uneventful except for a transient loss of consciousness possibly due to viral

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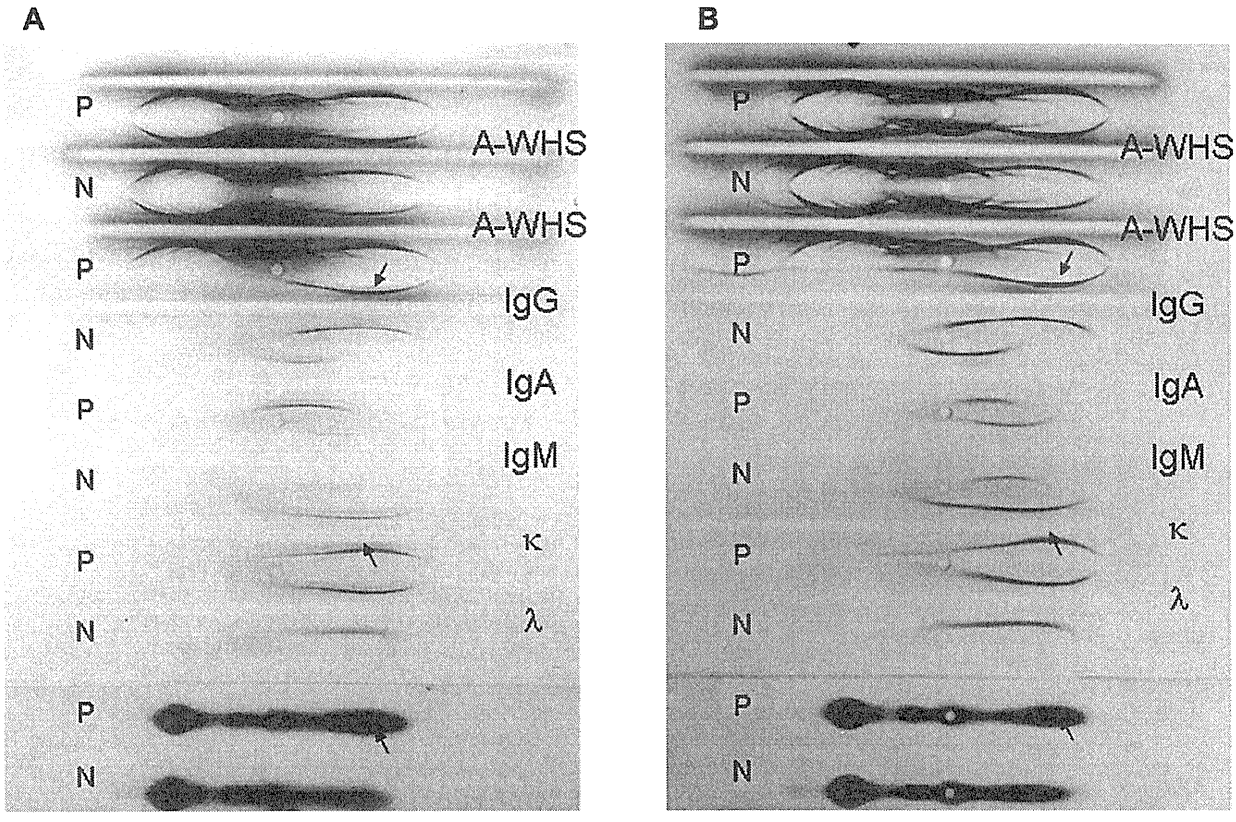

Figure 1. Immunoelectrophoresis before (**A**) and after (**B**) CBT. A moderate amount of IgG(κ)-type monoclonal protein was detected in both samples. λ = anti−λ-chain antibody; κ = anti−κ-chain antibody; A-WHS = anti-whole human serum antibody; IgA = anti−α-chain antibody; IgG = anti−γ-chain antibody; IgM = anti−μ-chain antibody; N = normal control serum; P = patient's serum.

encephalopathy. Neutrophil engraftment was achieved on day 16 after CBT. There were no signs of acute GVHD and cytomegalovirus reactivation. The serum IgG level was persistently under 1000 mg/dL (805 mg/dL on day 21 and 840 mg/dL on day 100).

MG became undetectable on day 33 after first BMT. Six months after CBT, however, routine blood test showed recurrence of MG without evidence of MDS relapse, although she had persistent anemia and thrombocytopenia. The pattern of immunoelectrophoresis after transplantation was similar to that before transplantation (IgG level, 2820 mg/dL; Fig. 1B). At 14 months after CBT, bicytopenia associated with elevation of serum lactate dehydrogenase level worsened. Bone marrow examination revealed blast cell count of 13% with trilineage morphological abnormalities. Cytogenetic analysis revealed clonal abnormalities, including 47, XY, +10, add(10)(p11.2)x2. Sex chromosome fluorescence in situ hybridization also showed 99.8% of XY cells. Therefore, we diagnosed donor-derived MDS. Subsequently, leukemic transformation was observed within a month and the patient died of leukemia despite chemotherapy and supportive treatments. The MG was persistently observed after relapse.

Genotyping

Genomic DNA was isolated using a QIAamp DNA mini kit (QIAGEN, Valencia, CA, USA) from the peripheral blood mononuclear cells and buccal mucosa of the patient. Genotyping of human immunoglobulin heavy chain allotype was performed as described previously [5]. Briefly, oligonucleotides PR1 (5′ CCCC TGGCACCCTCCTCCAA 3′) and PR2 (5′ GCCCTGGACTGGG GCTGCAT 3′) were used as the primer set for amplifying a 364-bp fragment from the constant region domain of the human IgG1 heavy chain. Conditions for polymerase chain reaction (PCR) amplification were as follows: 94°C × 2 minutes followed by 30 cycles of 96°C × 1 minute, 70°C × 30 seconds, 72°C × 1 minute, and final 7 minutes incubation at 72°C. Total volume of PCR mixture was 50 μL containing 1 μL genomic DNA, 1 mM $MgCl_2$, 5 U Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.2 mM dNTP mix, and 0.2 μM each primer. After separating the reaction products by 2% agarose gel electrophoresis and confirming a single-band pattern, all PCR products were purified using a QIAquick PCR Purification kit (QIAGEN) and sequenced in forward directions using an ABI 3700 instrument and BigDye sequencing kit (Applied Biosystems, Foster City, CA, USA).

Enzyme-linked immunosorbent assay for determination of IgG allotype IgG1m(f)

A 96-well microtiter plate (Nunc-Immuno Plate, Poly Sorp; Thermo Fisher Scientific, Rochester, NY, USA) was coated with 50 μL rabbit anti-IgG1m(f)1 antibody (I5385; Sigma, St Louis, MO, USA) diluted with phosphate-buffered saline (PBS) 1/2000 overnight at 4°C. After three washes with Tween 20 containing 0.05% PBS, the wells were blocked with 200 μL PBS with 2%

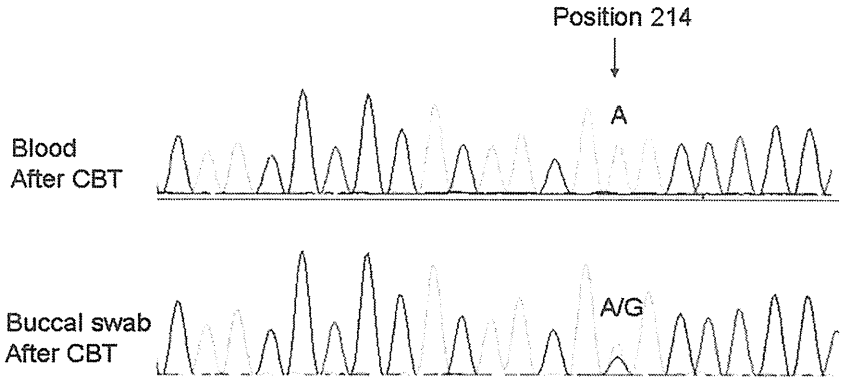

Figure 2. Genotyping of IgG1m (z) and (f) allotypes. A single base substitution of position 214 in γ1 chains of immunoglobulins in the coding DNA was examined. Genotyping of the patient's buccal mucosa was heterozygous for A and G, i.e., it showed an IgG1m(f,z) pattern, whereas her donor-derived peripheral blood mononuclear cells were homozygous for A, i.e., they showed an IgG1m(z) pattern.

fetal bovine serum (FBS) and incubated at room temperature for 1 hour. Then, 100 μL patient plasma diluted 1/1000 in PBS with 2% FBS was added to the wells in duplicate. The plasma samples from six healthy volunteers were used as positive (n = 3) and negative (n = 3) controls after confirming their genotypes. After incubation under continuous shaking at room temperature for 2 hours, the plate was washed three times. Next, 100 μL peroxidase-conjugated rabbit anti-human IgG whole molecule (A8792; Sigma) diluted 1/50,000 in PBS with 2% FBS was added and the plate was incubated for 1 hour at room temperature. After three washes, 100 μL ABTS peroxidase substrate system (KPL, Gaithersburg, MD, USA) was added to each well, and the plates were read by plate reader at 405 nm.

Chimerism analysis of T cells and B cells

After staining with fluorescein isothiocyanate–conjugated anti-human CD19 (BD Pharmingen, Franklin Lakes, NJ, USA) and phycoerythrin-conjugated anti-human CD3 (BD Pharmingen) cells were washed twice with PBS containing 5% FBS. Then, $CD3^-CD19^+$ B cells and $CD3^+CD19^-$ T cells were directly sorted into tubes using fluorescence-activated cell sorting Aria (BD Biosciences) and data were analyzed by FACS Diva software (BD Biosciences). Genomic DNA of each sorted cell was collected using a QIA Amp DNA Mini kit (QIAGEN) and chimerism analyses were performed by the short tandem repeat method as described previously [6]. Briefly, multiplex PCR was performed using primer sets (D5S818, D7S820, D13S317, and FGA; all labeled with FAM for recipient and with NED for donor). Separation and detection of the amplified PCR products were performed on an ABI 3130 genetic analyzer (Applied Biosystems) and results were analyzed using the GeneMapper V4.0 software (Applied Biosystems).

Results

Genotyping of IgG1m (z) and (f) allotypes

IgG1m(z) and (f) allotypes include lysine and arginine residues, respectively, at position 214 in the γ1 chain of immunoglobulins. This amino acid difference is due to a single base substitution in the coding DNA (A or G). Genotyping of our patient's buccal mucosa showed that her original IgG allotype was IgG1m (f,z), whereas her peripheral blood mononuclear cells at development of donor-derived MDS showed IgG1m(z) allotype (Fig. 2). In this study, positive result for IgGm(f) was defined as IgG1m(f,z) allotype (heterozygous for A and G at position 214), whereas negative result for IgGm(f) was defined as IgG1(z) allotype (homozygous pattern for A at position 214).

Enzyme-linked immunosorbent assay for determination of IgG allotype IgG1m(f) using patients' plasma after transplantation

In healthy volunteers of IgG1m(z) allotype [IgG1m(f)-negative controls], the absorbance average at 405 nm was <0.2 (range, 0.13–0.17), and it was >0.5 (range, 0.50–0.60) in volunteers of IgG1m (f,z) allotype [IgG1m(f)-positive controls]. The absorbance average of our patient was 0.17, same as IgG1m(z) pattern and we therefore concluded that she developed donor-derived MG (Fig. 3). Furthermore, the chimerism analyses of total peripheral blood mononuclear cells, B cells, and T cells revealed that all of these cells were of donor origin (data not shown).

Discussion

We examined a patient who developed donor-derived MDS after CBT for MDS. Recurrence of MG was observed before development of donor-derived MDS. We first considered that the MG was of recipient origin, as the recipient plasma cells may persist long after allogeneic hematopoietic stem cell transplantation. For example, pure red cell aplasia after allogeneic transplantation with ABO major mismatch is considered to be caused by persistent presence of recipient antibody against mismatched ABO antigens. In addition, thrombocytopenia caused by recipient-origin alloantibodies has been reported in BMT recipients [7,8]. One report described that antibodies of recipient-origin can be detected for up to 8 years because of the relative

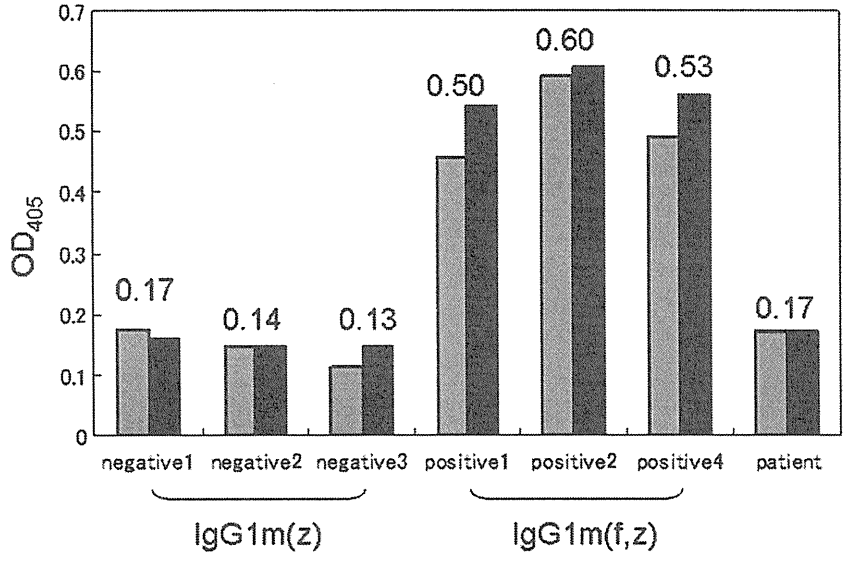

Figure 3. Enzyme-linked immunosorbent assay for determination of IgG allotypes. The absorbance average at 405 nm of IgG1m(z) normal volunteers was <0.2 (range, 0.13–0.17) [left, IgG1m(f)-negative], while that of normal volunteers of IgG1m (f,z) was >0.5 (range, 0.50–0.60) [right, IgG1m(f)-positive]. The absorbance average of our patient was 0.17, which was almost the same as that of IgG1m(z) volunteers.

chemotherapy and radiation resistance of recipient plasma cells. We performed Ig allotype analyses to identify the origin of MG after CBT. Although the allelic frequency of IgG1m(f,z) is almost only 10% in normal Japanese patients [9], we could identify Ig allotype gene polymorphisms in this patient because her original allotype was IgG1m(f,z). Contrary to our expectation, results of the analyses for Ig allotype showed that the MG after CBT was of donor origin.

MDS is known to be associated with significant immunologic disorders including abnormal production of gammaglobulin [1–4]. In 1986, Mufti et al. showed that patients with all categories of MDS had high prevalence (12.5%) of MG [2]. They also reported a high prevalence of both polyclonal hypergammaglobulinemia and hypogammaglobulinemia. In Japan, Okamoto et al. reported that hypergammaglobulinemia and hypogammaglobulinemia were observed in 39% and 8% patients, respectively, of 153 patients with MDS [10]. Dalamaga et al. showed that the incidence of hypergammaglobulinemia was high in MDS patients with cutaneous lesions, such as leukemia cutis, Sweet's syndrome, leukocytoclastic vasculitis, and photosensitivity [11]. A distinct subset of MDS patients also developed various types of autoimmune manifestations [12,13]. Interestingly, recent reports indicated that azacitidine treatment was effective for autoimmune symptoms in MDS patients [14].

The pathogenesis of immunological abnormalities in MDS patients remains poorly understood. Several hypotheses have been suggested as follows:

1. Impaired granulocyte function leading to chronic infection that persistently stimulates antibody production. With increasing rate of cell divisions in the B-lymphoid system, more genetic errors accumulate stochastically, leading to a high incidence of B-cell neoplasm [15].
2. It has been reported that an abnormal clone contains not only myeloid cells but also lymphoid cells in some MDS patients [16–18]. If a first-hit mutation occurred in the stem cells that could differentiate into mature myeloid and lymphoid cells, in the myeloid line, this would manifest as a feature of MDS and in the lymphoid line as types of immunological abnormalities.
3. In MDS, monocytes and macrophages are clearly derived from the abnormal clone. Although there is limited evidence about functional abnormalities of monocytes in MDS [19], abnormalities in monocyte/macrophage function may cause inappropriate secretion of interleukin-6 or other lymphostimulatory cytokines and increase the rate of B-cell division. In addition, some reports suggest that the number of dendritic cells (DCs), which are the professional antigen presenting cells (APCs), and their maturation state may be reduced in high-risk MDS [20,21]. The disruption of function of these APCs in MDS patients may lead to persistent immune stimulation either by poor clearance of bacterial antigens, overactive antigenic presentation, or unregulated cytokine secretion [4].

In our patient, MG in association with MDS developed repetitively before and after CBT. The prevalence of MG in MDS patients is 12.5%; however, in the absence of a background of MG, the probability of its recurrence in association with MDS is only 1% to 2%. If we hypothesize that the patient persistently had specific features that induced

development of MG before and after CBT, residual recipient's APCs might play a role in the pathogenesis of recurrent MG after CBT. Bogunovic et al. evaluated the chimerism of dermal DCs in mice and humans, and showed that a large proportion of recipient dermal DCs remain in recipient's skin even after achieving complete donor-type bone marrow chimerism after allogeneic transplantation [22]. Therefore, unlike peripheral recipient DCs, which are rapidly replaced by donor DCs, dermal recipient DCs are resistant to chemotherapy or radiotherapy in the conditioning regimen [23]. Especially, because our patient received a reduced-intensity conditioning regimen, it is very likely that the recipient's tissue DCs persisted after CBT, which induced recurrent immunoglobulin abnormalities. In addition, recipient's skin DCs can be eradicated by skin GVHD [24], but our patient did not develop GVHD after CBT.

Conclusions

We report a patient who developed recurrence of MG associated with donor-derived MDS after CBT for MDS with MG. Analyses of the IgG1 allotype gene polymorphisms identified the donor as the origin of the MG after CBT. Although the mechanism of the donor-derived MG remains unclear, persistent presence of recipient's APCs might have induced the abnormal immunoglobulin production.

Conflict of interest disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

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ORIGINAL ARTICLE

Immune recovery after autologous PBSC transplantation without *in vitro* graft manipulation for refractory systemic lupus erythematosus

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Autologous hematopoietic SCT (ASCT) has been investigated as salvage therapy for refractory systemic lupus erythematosus (SLE). Although immune recovery after ASCT with *in vitro* purging of lymphocytes has been extensively studied, little information is available about immune recovery after ASCT without *in vitro* purging. Therefore, we analyzed the immune recovery of a patient who successfully underwent ASCT without *in vitro* purging for refractory SLE. In addition to the numbers of PBL subsets, T-cell receptor rearrangement excision circles (TRECs) and the T-cell receptor repertoire diversity of both CD4+ and CD8+ T cells were sequentially analyzed. All SLE-related symptoms disappeared within 3 months after ASCT and the serum anti-dsDNA Ab became undetectable. The number of CD4+CD45RO+ memory T cells remained lower than that in healthy adult controls, but the number of CD4+CD45RA+ naïve T cells showed a rapid increase after ASCT. TRECs of both CD4+ and CD8+ T cells were strongly suppressed before ASCT, but consistently increased after ASCT. The T-cell receptor repertoire of CD8+ T cells was skewed before ASCT, but the diversity recovered after ASCT. ASCT with the reinfusion of a large number of autologous T cells did not impair the recovery of naive T cells or resetting of the immune system.

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease that may affect multiple organs, including the skin, joints, kidneys, nervous system and so on. Intensive immunosuppressive therapies, such as intermittent pulse of CY or mycophenolate mofetil, have been shown to improve the prognosis of patients with SLE. However, a subset of patients is refractory to these treatments and their clinical prognosis remains poor. Therefore, autologous hematopoietic SCT (ASCT) has been investigated as salvage therapy for refractory SLE. The procedure includes the collection of PBSCs following mobilization with CY and G-CSF, an immunoablative conditioning regimen using high-dose chemotherapy with or without antithymocyte globulin/antilymphocyte globulin (ALG), and the infusion of autologous PBSC with or without *in vitro* purging of lymphocytes. A single-center study by Burt *et al.* showed a 5-year overall survival of 84% and a 5-year disease-free survival of 50% after ASCT for patients with severe and treatment-refractory SLE.[1] The European Group for Blood and Marrow Transplantation (EBMT) Working Party on Autoimmune Disease analyzed all first ASCT for autoimmune diseases reported to the EBMT registry between 1996 and 2007.[2] Of the 900 patients analyzed, 85 had SLE and their 5-year progression-free survival was 44%. However, the procedure for ASCT significantly varied among centers. For example, antithymocyte globulin/ALG was used in 55% of patients, and intensive conditioning regimens, including TBI or BU, were applied in 10%. Especially, *in vitro* purging of lymphocytes was performed in 44% of patients. This strategy is based on the concept that the reinfusion of autologous lymphocytes may lead to the relapse of autoimmune disease. However, *in vitro* purging may also strongly affect the immune recovery after ASCT, whereas its impact on disease relapse remains unclear.[2]

The delayed immune recovery after ASCT for autoimmune disease has been associated with infectious complications.[3] Although immune recovery after ASCT with *in vitro* purging has been extensively studied,[4,5] little information is available about immune recovery after ASCT without *in vitro* purging. Therefore, in this study, we analyzed the immune recovery of a patient who

successfully underwent ASCT without *in vitro* purging for refractory SLE.

Materials and methods

Cell preparation
PBMCs were isolated from peripheral blood by Ficoll-hypaque density centrifugation. CD4+ and CD8+ T cells were purified using the MACS magnetic bead separation system (Miltenyi Biotec, Auburn, CA, USA).

T-cell receptor rearrangement excision circle quantification
T-cell receptor rearrangement excision circles (TRECs) were quantified in purified CD4+ and CD8+ T cells by real-time quantitative PCR with the 5′-nuclease (TaqMan) assay and an ABI7900 system (Life Technologies, Foster City, CA, USA) as described elsewhere.[6] Briefly, cells were lysed in 100 μg/mL proteinase K for 1 h at 56 °C, followed by 10 min at 95 °C at 107 cells/mL. Next, real-time quantitative PCR was carried out on 2.5 μL cell lysate under the following conditions: 50 °C for 2 min, followed by 95 °C for 10 min, and finally 40 cycles of amplification (95 °C for 15 s and 60 °C for 1 min). The sequences of the primers and probe were as follows: forward primer 5′-CAC ATCCCTTTCAACCATGCT-3′, reverse primer 5′-GCCA GCTGCAGGGTTTAGG-3′, and the probe 5′-FAM-ACA CCTCTGGTTTTTGTAAAGGTGCCCACT-TAMRA-3′. A standard curve was plotted, and TREC values for samples were calculated by ABI PRISM 7900 software. Cell lysates were checked for the consistency of DNA content by an internal control gene. The mean copy numbers of TRECs in 10^5 CD4+ and CD8+ T cells from 10 healthy adults were 1440 ± 880 and 1920 ± 1300, respectively.

T-cell receptor repertoire analysis
T-cell receptor repertoire analysis has been described elsewhere.[7] Briefly, total RNA was purified by a QIAamp RNA blood Mini Kit (Qiagen, GmbH Germany). The RNA was then reverse transcribed into cDNA using random primers and SuperScript II reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was PCR amplified under the following conditions: 95 °C for 10 min, followed by 35 cycles (94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min) with a primer specific to 24 different BV subfamilies (BVs 1–203 and BVs 21–244) and a fluorescent BC primer.[8,9] In all, 1 μL of amplified products was mixed with 20 μL HiDi formamide and 0.5 μL GS500 ROX size standard, and the mixture was electrophoresed by an ABI 3100 system. Data were analyzed with GeneMapper software (Life Technologies).

The overall complexity within a Vβ subfamily was determined by counting the number of discrete peaks and determining their relative size on the electropherogram by a complexity scoring system as described elsewhere.[10]

Complexity score = (total peak area/sum of the major peak areas) × (number of major peaks). Major peaks were defined as peaks whose area was at least 10% of the total peak area.

The mean complexity scores of CD4+ and CD8+ T cells from 10 healthy adults were 116.1 ± 2.36 and 109.49 ± 6.77, respectively.

Case report
A 19-year-old female developed oral ulcers, polyarthritis and a butterfly-like rash. She was diagnosed to have SLE. Her symptoms, including high fever, rash, oral mucositis and polyarticular joint pain, showed only transient improvement in response to various treatments including high-dose steroids (that is, betamethasone pulse at 100 mg/day for 3 days), CYA (150 mg/day), tacrolimus (4 mg/day), pulsed CY (500–800 mg for eight times), rituximab (500 mg twice), high-dose i.v. gammaglobulin and plasma exchange during the clinical course of ~4 years. She also developed lupus nephritis and central nervous system manifestations. Therefore, we planned ASCT after approval by the ethics committee and after obtaining written informed consent from the patient.

At this time, she was 22 years old. She had persistent fever between 37.5 and 39 °C, a butterfly-like rash and oral mucosal ulcers. Articular pain was observed at the elbow, wrist, hip, knee and ankle. The WBC count was 7.97×10^9/L with 85% neutrophils and 14% lymphocytes. The hemoglobin level was 8.7 g/dL and the platelet count was 466×10^9/L. The serum anti-dsDNA IgG Ab was detected at 413 U/mL (normal range: <10 U/mL). Serum complement levels of C3 and C4 were decreased to 70 mg/dL (normal range: 65–135 mg/dL) and 8 mg/dL (normal range 13–35 mg/dL), respectively. She required oral betamethasone at 7.5 mg/day to alleviate these symptoms.

PBSCs were mobilized with the administration of CY at 2 g/m^2 followed by G-CSF (filgrastim at 600 μg/day). A sufficient number of CD34+ cells was obtained by apheresis and cryopreserved without graft manipulation. The conditioning regimen before ASCT included the i.v. administration of CY at 50 mg/kg/day from day −6 to day −3 and rabbit ALG (Zetbulin, Fresenius, Munich, Germany) at 5 mg/kg/day from day −5 to day −2. After the infusion of autologous peripheral blood graft including CD34+ cells at 2.27×10^6/kg and CD3+ cells at 0.22×10^8 cells/kg, G-CSF (filgrastim at 75 μg/day) was started on day 7 after ASCT. Neutrophil recovery of more than 0.5×10^9/L was observed on day 10. The dose of betamethasone was gradually decreased from 7.5 to 3 mg/day during these procedures.

With regard to the disease activity of SLE, arthritis and butterfly erythema began to improve after we administered CY to mobilize PBSC, and thus we started tapering the dose of steroid. After ASCT, the anti-dsDNA Ab became undetectable (Figure 1a). The SLE disease activity index 2000 (SLEDAI-2K) score was 15 before the administration of CY to mobilize PBSC, but decreased to 4 at 30 days after ASCT, and all the SLE-related symptoms disappeared (SLEDAI-2K score 0) within 3 months after ASCT.[11] She returned to nursing school and is currently in good physical condition after 2 years of ASCT. The dose of steroid is

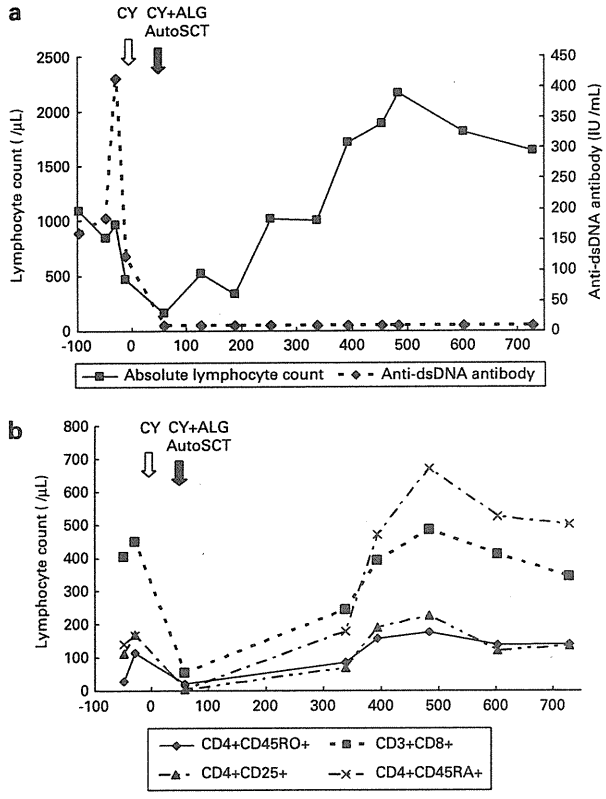

Figure 1 (a) Serial changes in the lymphocyte count and anti-dsDNA Ab level. (b) Recovery of CD4 + CD45RO + memory T cells, CD4 + CD45RA + naive T cells, CD4 + C25 + T cells and CD8 + T cells. ALG = antilymphocyte globulin; AutoSCT = autologous hematopoietic SCT.

being gradually tapered, and the last prescribed dose was 8 mg/day of prednisolone.

Immune recovery

The PBL count remained lower than 0.5×10^9/L in the first 6 months after ASCT, but was persistently higher than 1.0×10^9/L over the next 18 months, whereas the anti-dsDNA Ab level was sufficiently suppressed (Figure 1a). The results of immunophenotypic analyses are shown in Figure 1b. The number of CD4 + CD45RO + memory T cells remained lower than that in the healthy adult controls (400/μL).[4] On the other hand, the number of CD4 + CD45RA + naïve T cells showed a rapid increase between 12 and 18 months after ASCT, and became higher than that in the healthy controls (300/μL). As shown in Table 1, TRECs of both CD4 + and CD8 + T cells were strongly suppressed before ASCT (156 and 356 copies/10^5 cells, respectively), but were consistently increased after ASCT. At 2 years after ASCT, TRECs of CD4 + and CD8 + T cells were 2602 and 1584 copies/10^5 cells, respectively, and were almost equivalent to those in healthy adults (1440 ± 880 and 1920 ± 1300, respectively).

With regard to T-cell receptor repertoire analyses, the T-cell receptor repertoire of CD4 + T cells was diverse before ASCT (Table 1). Repertoire skewing was observed after ASCT, but the diversity was reconstituted 1 year after

Table 1 Evaluation of TREC and T-cell receptor repertoire complexity after autologous PBSC transplantation

Day	*TREC*		*T-cell receptor repertoire complexity*	
	CD4	*CD8*	*CD4*	*CD8*
−7	156	356	124.11	94.37
337	1450	556	94.93	120.79
393	1578	986	121.19	112.3
484	1934	1374	123.35	110.02
729	2602	1584	124.88	121.09
Control	1440 ± 880	1920 ± 1300	116.1 ± 2.36	139.49 ± 6.77

Abbreviations: Control = mean value in 10 healthy adults; TREC = T-cell receptor rearrangement excision circle.

ASCT. On the other hand, the T-cell receptor repertoire of CD8 + T cells was skewed before ASCT (Figure 2a). However, the diversity recovered 2 years after ASCT (Figure 2b).

The number of CD4 + CD25 + T cells was decreased after ASCT, but recovered to the pre-ASCT level 1 year after ASCT. The kinetics of the number of CD8 + cells was similar. We did not evaluate the expression of intracellular FoxP3, and thus the exact number of regulatory T cells was not determined. However, it has been reported that between one- and two-thirds of CD4 + CD25 + T cells were positive for FoxP3 in SLE patients after ASCT.[4] Therefore, the number of FoxP3 + regulatory T cells at 1–2 years after ASCT was expected to be, at least, equivalent to that in healthy controls (65/μL).[4]

The serum IgG level was 9.92 g/L before ASCT and decreased to 5.62 g/L at 7 months after ASCT, but has remained consistently higher than 10 g/L beginning 1 year after ASCT. Serum IgA and IgM levels were persistently within the normal range.

Discussion

The mechanism of action of ASCT includes not only the elimination of autoreactive effector and inflammatory cells by an immunoablative conditioning regimen but also resetting of the adaptive immune system.[12,13] The reactivation of thymic function after ASCT was illustrated by an increase in the number of CD31 + CD45RA + CD4 + T cells and the amount of TRECs.[12,13] T-cell receptor repertoire analysis revealed improved diversity after ASCT.[12,13] In addition, the regeneration of thymic-derived FoxP3 + regulatory T cells may contribute to the induction of immune tolerance.[4,14] However, these analyses of immune recovery were performed in patients who underwent ASCT with *in vitro* purging of lymphocyte.

We did not perform *in vitro* purging in the current patient, as *in vitro* purging may delay the immune recovery after ASCT and induce infectious complications.[3] On the other hand, it is not clear whether the relapse rate after ASCT is decreased by *in vitro* purging.[2] This patient remains relapse free after 2 years of ASCT. Therefore, the efficacy of ASCT may not be spoiled by omitting *in vitro* purging, at least, in this patient, although the follow-up duration is still too short to draw definitive conclusions.

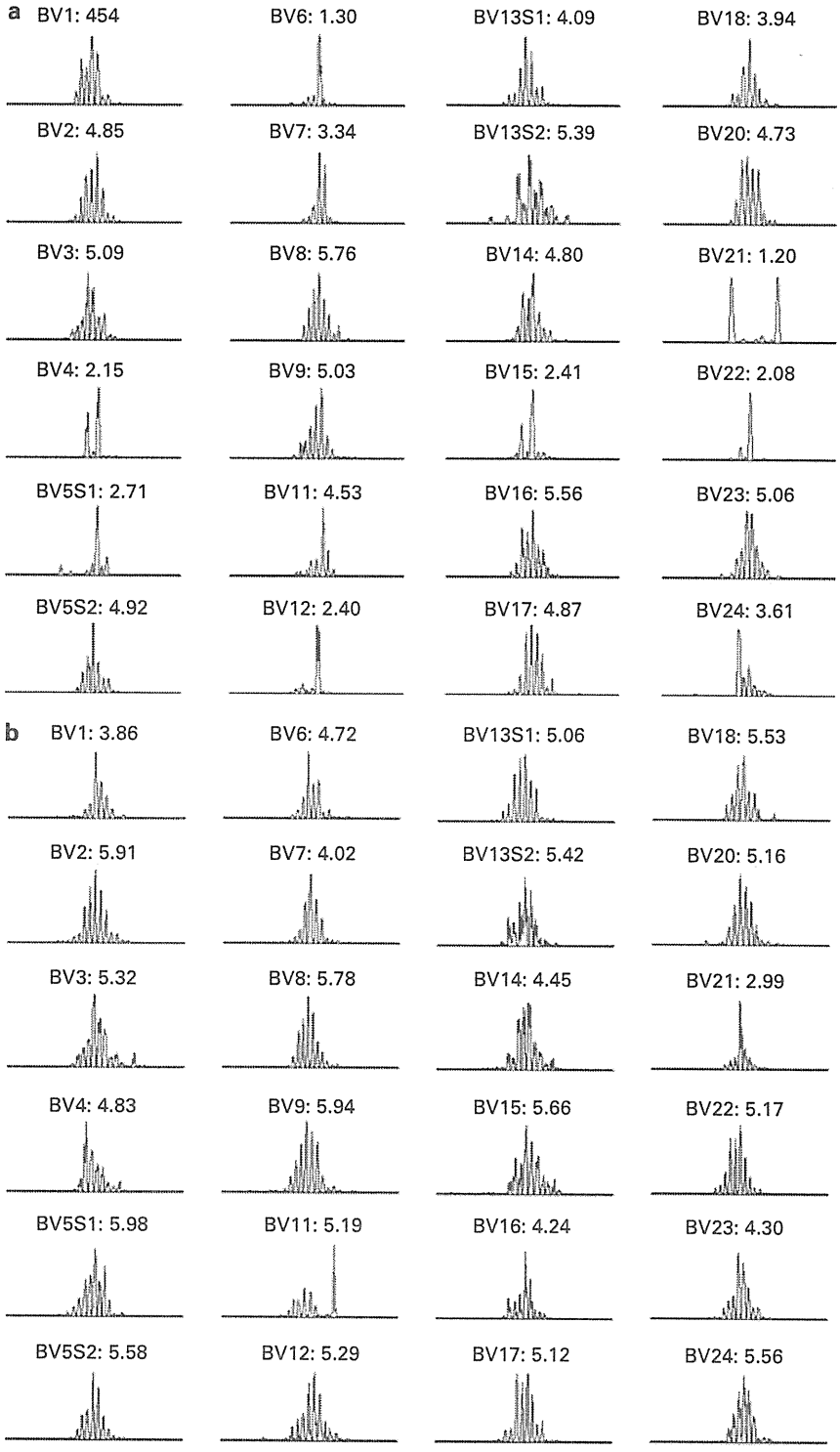

Figure 2 T-cell receptor repertoire analyses of CD8+ T cells before (**a**) and after (**b**) ASCT.

With regard to immune recovery, we compared the data for this patient who received unmanipulated graft with those in seven patients who underwent ASCT with *in vitro* purging in the detailed immune recovery analyses by Alexander *et al.*[4] The number of infused CD34+ cells was similar: 2.27×10^6 cells/kg in this patient vs 2.0–6.1×10^6 cells/kg in the seven patients. However, the number of infused CD3+ cells in this patient (0.22×10^8 cells/kg) was much higher than that in the previous study ($0.4–1.6 \times 10^4$ cells/kg). The brand of ALG was the same,

but the total dose of ALG in the conditioning regimen was lower in the current patient (25 vs 90 mg/kg). This difference might have also affected the immune recovery after ASCT, as ALG administered before ASCT depletes T cells not only in the patient but also in the infused graft.

The recovery of CD4 + CD45RO + memory T cells was similar, but that of CD4 + CD45RA + naïve T cells in this patient was faster than that in the previous study (median of 503 vs 244/μL at 2 years after ASCT). The significant recovery of TRECs might contribute to the fast reconstitution of naïve T cells. In addition, T-cell receptor repertoire diversity of CD8 + T cells was improved after ASCT, although the diversity of CD4 + T cells was not impaired even before ASCT. Such reconstitution of the juvenile immune system may have contributed to the reestablishment of tolerance.

In conclusion, in this single patient, ASCT with the reinfusion of a large number of autologous T cells by omitting *in vitro* purging did not impair the recovery of naive T cells or resetting of the immune system compared with ASCT with *in vitro* purging. Infused T cells might have prevented severe infectious complications early after ASCT. The present patient is in clinical remission after 2 years of ASCT. However, a larger study is required to confirm the benefit of ASCT without *in vitro* purging.

Conflict of interest

The authors declare no conflict of interest.

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LETTER TO THE EDITOR

Risks and benefits of ovarian shielding in female patients undergoing TBI: a decision analysis

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In a recent issue of *Bone Marrow Transplantation*, Courbiere *et al.*[1] raised the possibility of leukemia relapse after autologous transplantation of cryopreserved ovarian tissue from leukemic cell contamination in the graft. They detected a small copy number of Bcr–Abl transcripts by RQ-PCR in the ovarian tissue from an 18-year-old woman with CML. We agree that ovarian transplantation could be proposed (once patients are informed of the risk of leukemia relapse), as there were few contaminating leukemic cells and a GVL effect may be protective.

In Japan, cryopreservation of ovarian tissue is not available, but two other strategies are used to preserve fertility in young women undergoing hematopoietic SCT. One is embryo or oocyte cryopreservation for women with or without a partner, respectively. Although the success rate after transfer of thawed fertilized oocytes had been low previously, both post-thaw survival and fertilization rates of frozen oocytes[2,3] have improved. Nonetheless, concern remains regarding the potential for chromosomal aneuploidy or other karyotypic abnormalities in the offspring, as cryopreservation may affect the meiotic spindle of oocytes.[4] In addition, it is generally difficult to obtain good-quality oocytes from patients receiving chemotherapy.[5]

Another strategy is ovarian shielding in women undergoing TBI. Whereas ovarian recovery is observed in only 10–15% of patients receiving standard conditioning with CY and TBI,[6] most patients show ovarian recovery after high-dose CY alone.[6] Ovarian function can therefore be preserved by reducing the radiation dose to the ovaries. We previously reported that ovarian function was recovered in about 80% of patients who underwent ovarian shielding.[7,8] The incidence of leukemia relapse may not increase if this procedure is performed in patients in remission, as the total radiation dose to the ovaries was approximately 3 Gy in this protocol, which is higher than the TBI dose (2 Gy) in the non-myeloablative regimen of the Seattle group associated with a relapse rate similar to that of a myeloablative regimen.[9] However, a large number of patients is required to determine the actual change in the incidence of relapse under ovarian shielding.

To overcome the difficulty for both physicians and patients in deciding whether or not to perform ovarian shielding, we have used a decision analysis approach. We constructed a decision tree using TreeAge Pro 2009 software (Williamstown, MA, USA) (Figure 1). The square at the left represents a decision node. We can decide either to perform ovarian shielding or not. Circles represent chance nodes and each chance node has 2 or 3 possible outcomes with a specific probability, called the transition probability. Every branch finally ends with triangles, called terminal nodes, and each terminal node has an assigned payoff value, called utility, according to different health states. Calculations were performed backward, from right to left in the decision tree. The sum of the products of transition probabilities and the utilities of the branches becomes the expected value for each chance node, and eventually the sum of the expected values in all of the chance nodes following the decision nodes becomes the expected value of each decision. To make a simple decision model, we determined the transition probabilities based on data from patients who underwent allogeneic transplantation for acute leukemia in first remission. The incidences of transplant-related mortality and relapse were assumed to be 0.2 (20%).[10] However, the incidence of relapse may increase with ovarian shielding ('relapse after ovarian shielding' in Figure 1). Therefore, while the cure rate is '1–0.2–0.2 = 0.6 (60%)' after a decision to not perform ovarian shielding, it is '1–0.2—relapse after ovarian shielding' after a decision to perform ovarian shielding. The probability of ovarian recovery was determined to be 10% after a decision to not perform ovarian shielding and 80% after a decision to perform ovarian shielding based on the literature.[6,7] Each patient's view of life can be reflected in the value of 'alive without ovarian recovery'. Under the simple assumption that the payoff values of transplant-related mortality and relapse were both 0 points and the payoff value of cure with ovarian recovery is 100 points, each patient can score the payoff value for 'alive without ovarian recovery' based on her own view of life. Patients for whom ovarian recovery is very important assign a low payoff value for 'alive without ovarian recovery'.

The expected values for the decisions vary according to the values of 'relapse after ovarian shielding' and 'alive without ovarian recovery'. For example, if we fix the value of 'relapse after ovarian shielding' at 30% under the assumption that the incidence of relapse is increased by 10% under ovarian shielding, the expected values for the two decisions vary according to the value of 'alive without ovarian recovery', as shown in Figure 2a (one-way sensitivity analysis). The expected value for a decision to not perform ovarian shielding is higher than that to perform ovarian shielding when a patient scores 'alive without ovarian recovery' higher than 77.3 points. If we fix the value of 'relapse after ovarian shielding' at 40%, the expected value for a decision to not perform ovarian

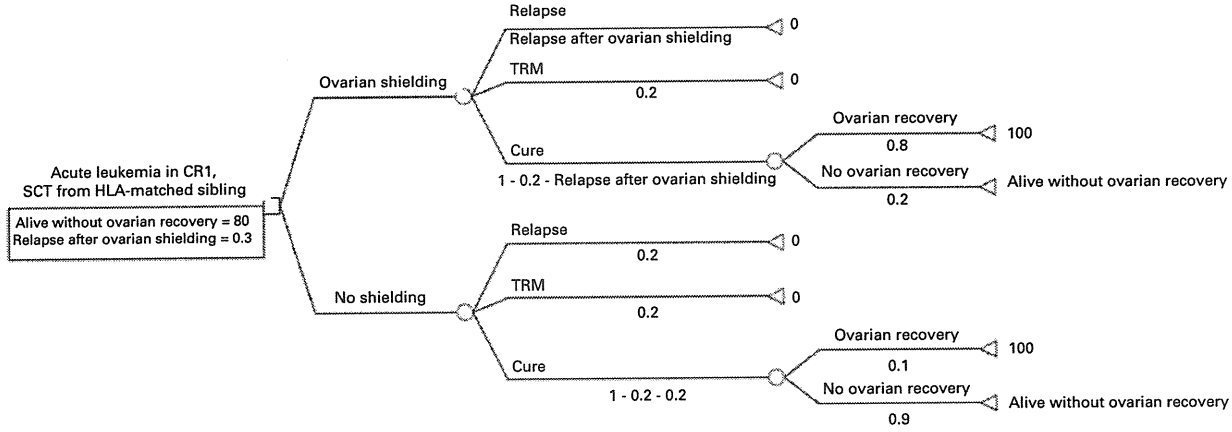

Figure 1 The decision tree used in this decision analysis.

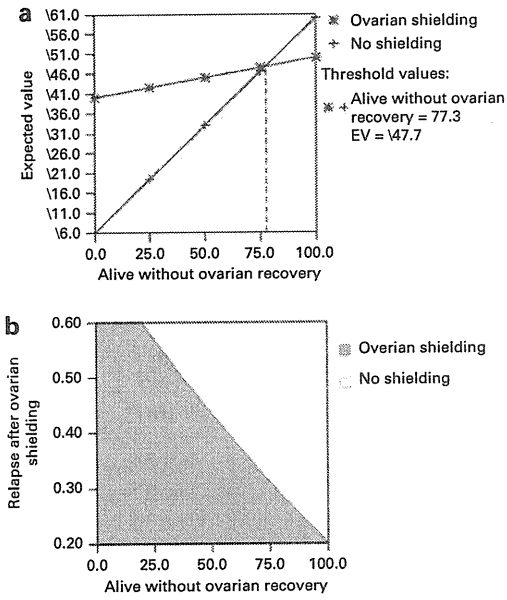

Figure 2 One-way (**a**) and two-way (**b**) sensitivity analyses. In the gray area, the expected value of a decision to perform ovarian shielding is higher than that of a decision to not perform ovarian shielding.

shielding is higher than that to perform ovarian shielding when a patient scores 'alive without ovarian recovery' higher than 56.5 points.

These two values can be changed simultaneously, as shown in Figure 2b (two-way sensitivity analysis). The threshold of the relapse rate, at which there is a change in which decision is made, can be obtained by drawing a vertical line from the 'alive without ovarian recovery' value for each patient. For example, if a patient scores 50 points for the payoff value of 'alive without ovarian recovery', the expected value for a decision to not perform ovarian shielding is higher than that to perform ovarian shielding when 'relapse after ovarian shielding' is higher than 43%, as the vertical line from the X-axis at 'alive without ovarian recovery' of 50 points crosses the borderline of the gray and white areas at 'relapse after ovarian shielding' of 43%.

Although this decision analysis is not definitive, it may be helpful for patients who find it difficult to make a decision when faced with uncertainty. Some young female patients tend to overestimate the value of fertility in their subsequent life, and we should inform patients that they can become pregnant using a donated oocyte even after their ovarian function is lost.

Conflict of interest

The authors declare no conflict of interest.

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Allogeneic stem cell transplantation as treatment for heavily treated, refractory acute graft-versus-host disease after HLA-mismatched stem cell transplantation

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Objective. **No effective treatment has been established for patients with steroid-refractory acute graft-versus-host disease (GVHD). Recently, we demonstrated in a murine tandem bone marrow transplantation model that life-threatening GVHD established by the first bone marrow transplantation was successfully treated by engraftment of a second donor graft after reduced-intensity conditioning. We named the effect by which allografts counteract GVHD "graft-versus-GVHD."**

Materials and Methods. **To investigate the efficacy of graft-versus-GVHD treatment clinically, 16 patients who developed, after human leukocyte antigen–mismatched stem cell transplantation, severe GVHD, refractory to three to five lines of GVHD-specific treatments, underwent 17 allogeneic stem cell transplantations using reduced-intensity conditioning regimens with grafts from a second donor.**

Results. **Among the 15 transplantations that could be evaluated, rescue donor grafts were engrafted in 11 cases and rejected in 4 cases. For patients who achieved rescue donor engraftment, the response rate was 90.9% (eight complete response, two partial response, and one stable disease). Six of the eight patients with complete response survived without GVHD symptoms, with a median follow-up of 2128 days. No new development of GVHD by the second graft was observed. No patients had recurrence of the original malignant disease. In contrast, no long-term survivors were observed in patients who rejected rescue donor grafts.**

Conclusions. **We propose here a novel graft-versus-GVHD treatment to treat refractory GVHD, and these results strongly suggest that GVHD can be successfully treated by eliminating the harmful lymphocytes responsible for GVHD by a second allogeneic stem cell transplantation.**

Graft-versus-host-disease (GVHD) is a major obstacle to successful allogeneic bone marrow transplantation (BMT), and greatly limits the applications and efficacy of allogeneic BMT. In particular, for steroid-refractory GVHD, no consensus treatment has been established [1,2], although a number of therapeutic approaches, including mesenchymal stem cells, pentostatin, infliximab, and a variety of monoclonal antibodies, have been reported [3–7].

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We and others have attempted to treat patients with severe GVHD by second transplantation using autologous or syngeneic hematopoietic cells to ablate the lymphoid cells responsible for GVHD [8–10]. Although severe GVHD resolved or partially improved after these transplantations, relapse of the original tumor occurred in the majority of patients.

Therefore, we intended to use a second allogeneic donor as a graft source for rescue transplantation against GVHD. We recently demonstrated in a murine tandem BMT model where the three mouse strains shared one major histocompatibility complex haplotype and the other major histocompatibility complex haplotype was different, that

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life-threatening GVHD established by the first BMT using myeloablative conditioning was successfully treated by engraftment of a second donor graft using reduced-intensity conditioning treatment [11]. In allogeneic stem cell transplantation (SCT) for autoimmune diseases, donor lymphocytes are considered to have the capacity to eliminate all residual self-reactive host lymphocytes through a process known as graft-versus-autoimmunity effects [12], with analogy to graft-versus-leukemia (GVL) in leukemia. Thus, we named the effects by which second allografts counteract GVHD through permanent elimination or transient reduction of first donor harmful lymphocytes, "graft-versus-GVHD" [11].

In addition, clinically, we recently developed a novel unmanipulated human leukocyte antigen (HLA)–haploidentical nonmyeloablative SCT using a conditioning treatment consisting of fludarabine + busulfan + anti–T-lymphocyte globulin (ATG), and GVHD prophylaxis consisting of tacrolimus (FK506) + methylprednisolone (mPSL) (1 mg/kg), in which the incidence of acute GVHD was only 20% [13]. As some GVHDs occurred after donor lymphocyte infusion or rapid tapering of immunosuppressive agents for early relapse or severe viral infections, the actual incidence of GVHD was estimated to be 10%; therefore, we applied this HLA-haploidentical nonmyeloablative SCT to rescue transplantation for refractory GVHD.

In the present study, we investigated whether second allogeneic SCT could treat patients with severe, steroid-refractory GVHD.

Materials and methods

Patients

From February 2001 to December 2008, 320 patients underwent allogeneic SCT at Osaka University Hospital or at the Hospital of Hyogo College of Medicine. Among them, 16 consecutive adult patients who developed severe refractory GVHD after HLA-mismatched SCT underwent a second allogeneic SCT to treat GVHD. All of these patients were in remission at the time of rescue transplantation. The major objectives in this study were improved GVHD and survival at 6 months. GVHD was diagnosed from a biopsy of at least one involved organ. Patients with severe GVHD (≥grade II) who did not respond to mPSL (≥2 mg/kg) or who had recurrent GVHD at a dose of steroids ≥1 mg/kg mPSL were eligible for the study; however, patients who were finally enrolled received a median of four (range of two to five) lines of GVHD-specific treatments, including tumor necrosis factor blocker, ATG, and mycophenolate mofetil, by the time of the rescue transplantation (Table 1). In general, GVHD occurring after HLA-mismatched SCT progresses very rapidly, and quickly becomes irreversible; therefore, in the first SCT inducing GVHD, when the manifestations of GVHD worsened during 3 days of treatment, other immunosuppressive agents were added [14], sometimes in combination. Regarding the eligibility criteria for the rescue transplantation, patients who had HLA-identical or HLA 1–3 antigen-mismatched related donors were eligible. Patients were not eligible for rescue transplantation if they had severe renal, heart, or lung disease: serum creatinine level >1.5 times the normal upper limit, ejection fraction <50% on an echocardiogram, or oxygen saturation <93%, respectively. Patients were not eligible for rescue transplantation if they had severe liver disease that was considered to be caused by diseases other than GVHD; total bilirubin level >2.0 mg/dL, and aspartate aminotransferase >2.5 times the normal upper limit.

The characteristics of the patients and first transplantation inducing severe GVHD are shown in Table 1. Because one patient underwent allogeneic rescue SCT twice, 17 graft-versus-GVHD treatments were performed. Among the 16 patients, 14 had developed acute GVHD after allogeneic SCT, including 3 patients who had developed recurrent acute GVHD >100 days after transplantation and 2 after donor lymphocyte infusion. Institutional review board approval was obtained for the treatment protocol, and written informed consent was obtained from the patients and their families.

Four patients underwent the first transplantation (inducing severe GVHD) using a graft from an HLA 2–3 antigen-mismatched donor, and underwent the second (rescue) transplantation using a graft from an HLA-matched or 1 antigen-mismatched donor (Table 2). The donor in the first transplantation was selected for the following reasons. We recently reported that unmanipulated HLA-haploidentical SCT was useful for treating patients with hematologic malignant diseases in the advanced stage [13,15,16]. Thus, in our HLA-haploidentical SCT protocol, patients with a full-blown relapse can undergo allogeneic SCT using a graft from an HLA-haploidentical donor, even when an HLA-matched (or 1 antigen-mismatched) related donor is available. Such decisions were made at the recommendation of the physicians and with the concurrence of the patient and family members after considering the overall risks of recurrent malignancy, graft rejection, and severe GVHD with the two different types of donors.

Rescue transplantation procedure

Details of the rescue transplantation are shown in Table 2. Median interval between the previous allogeneic SCT and the rescue transplantation was 59 days (range, 32–481 days). All patients received a reduced-intensity conditioning treatment. The conditioning consisted of 30 mg/m^2 fludarabine intravenously for 3 consecutive days on days −6 to −4, ATG (Fresenius) 2 mg/kg/day for 4 days (day −4 to day −1) with or without total body irradiation 3 Gy on day 0. Eight patients could not receive total body irradiation because they had received total body or local irradiation as previous treatments. One patient (no. 10–2) who rejected the first rescue transplantation received thiotepa 10 mg/kg on day −2 and total body irradiation 4 Gy on day −1 in addition to fludarabine and ATG. In all cases, peripheral blood stem cells were used as the stem cell source.

GVHD prophylaxis was performed with FK506 and mPSL (1 mg/kg), as reported previously [13]. In brief, FK506 treatment was initiated the day before transplantation and given at a dose of 0.02 mg/kg/day as a continuous infusion. The target blood concentration of FK506 was set between 8 and 10 ng/mL until day 30, and was thereafter tapered in the absence of acute GVHD. Patients received intravenous FK506 therapy until they could reliably receive oral medications after transplantation. Intravenous administration of mPSL was started at a dose of 1 mg/kg/day from day −4. mPSL tapering was started in the third week and was performed relatively rapidly until day 30 using the serum soluble

Table 1. Patients' characteristics and first transplantation inducing severe GVHD

No	Sex/Age	Disease	Disease status	Conditioning regimen	Donor	HLA disparity	PS	grade	Stage			prior treatment for GVHD
									skin	gut	liver	
1	23/F	ALL	PR	full	Mother	2/2†	50	II	3	1	0	MTX, MMF, mPSL(2), Flu,
2	17/M	LBL	Re3	full	Cousin	2/3	10	III	3	3	1	Flu, ATG, MTX, MMF(inc)
3	33/M	ALL	PR	full	Sibling	3/3	20	III	3	4	0	MTX, MMF(inc), Flu, ATG,
4	37/M	MDS	RAEB	full	Offspring	3/3	20	III	3	4	0	Flu, MMF(inc), infliximab, ATG, pulse mPSL
5	25/M	CML	Re(autoBM)*	full	Sibling	2/2	70	II	3	0	0	PSL(inc). MMF
6	21/F	NHL	CR2(autoPB)	full	Mother	2/0	50	II	3	0	0	MMF, infliximab
7	19/M	HD	RR	full	Father	3/2	50	IV	4	0	0	MTX, ATG, infliximab
8	22/M	ALL	Re2	full	Sibling	3/2	10	III	0	3	3	infliximab, ATG, pulse mPSL, MTX, basiliximab
9	19/F	CML	BC	full	Sibling	2/2	70	II	3	0	0	infliximab, ATG, pulse mPSL, MTX, MMF(inc)
10-1	19/M	SNCL	IF	full	Sibling	2/2	50	III	3	4	0	MTX, infliximab, pulse mPSL, ATG
10-2	19/M	SNCL	IF	RIST	Mother	2/2	30	III	3	2	0	mPSL(inc), infliximab, MMF, ATG
11	41/F	LAHS	IF	full	Offspring	2/3	20	III	3	2	3	ATG, infliximab, MMF, pulse mPSL
12	21/F	AML	Re(alloBM)	RIST	Father	3/3	20	III	2	2	2	infliximab, pulse mPSL, MMF, ATG
13	49/M	CML	CP	RIST	Offspring	2/3	30	IV	4	2	0	ATG, MTX, infliximab, pulse mPSL
14	19/F	ALL	Re2	full	Sibling	3/1	40	III	2	3	1	pulse mPSL, MMF, etanercept, ATG
15	47/F	ALL	Re(alloPB)	RIST	UCB	4/2	40	III	3	3	0	etanercept, MMF, pulse mPSL
16	31/F	ALL	RR	full	Sibling	3/2	60	III	2	2	0	PSL(inc), pulse mPSL, MTX

AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; HD, Hodgkin's lymphoma; SNCL, small non-cleaved lymphoma; LAHS, lymphoma-associated hemophagocytic syndrome; CR2, second complete remission; PR, partial remission; Re, relapse; Re2 or Re3, second or third relapse; RR, resistant relapse; RAEB, refractory anemia with excess of blasts; CP, chronic phase; BC, blastic crisis; IF, induction failure; full, full regimen; RIST, reduced intensity of conditioning treatment; PS, Karnofsky performance status; MTX, methotrexate; MMF, mycophelate mofetil; mPSL(2), methylprednisolone 2 mg/kg; pulse mPSL, pulse therapy of methylprednisolone; Flu, fludarabine; ATG, anti-T-lymphocyte globulin; inc, increase in dose; autoBM, autologous bone marrow transplantation; autoPB, autologous peripheral blood stem cell transplantation; alloBM, allogeneic bone marrow transplantation; alloPB, allogeneic peripheral blood stem cell transplantation.

*Transplantation in parentheses indicates previous stem cell transplantation.

†Numbers before or after a slash indicate mismatched HLA antigens in GVH or HVG directions, respectively.