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References

1. Mohty M, Labopin M, Volin L, et al. Reduced-intensity versus conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. *Blood.* 2010;116(22):4439-4443.
2. Ottmann OG, Pfeifer H. Management of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). *Hematology Am Soc Hematol Educ Program.* 2009;371-381.
3. Cho BS, Lee S, Kim YJ, et al. Reduced-intensity conditioning allogeneic stem cell transplantation is a potential therapeutic approach for adults with high-risk acute lymphoblastic leukemia in remission: results of a prospective phase 2 study. *Leukemia.* 2009;23(10):1763-1770.
4. Stein AS, Palmer JM, O'Donnell MR, et al. Reduced-intensity conditioning followed by peripheral blood stem cell transplantation for adult patients with high-risk acute lymphoblastic leukemia. *Biol Blood Marrow Transplant.* 2009;15(11):1407-1414.
5. Marks DI, Wang T, Perez WS, et al. The outcome of full intensity and reduced intensity conditioning matched sibling or unrelated donor (URD) transplantation in adults with Philadelphia chromosome negative acute lymphoblastic leukemia (PH-ALL) in first and second complete remission (CR1 and CR2). *Blood.* 2010;116(3):366-374.
6. Allison PD. *Missing Data.* Thousand Oaks, CA: Sage Publications; 2001.

To the editor:

Dysregulation of the HIF pathway due to *VHL* mutation causing severe erythrocytosis and pulmonary arterial hypertension

Hereditary erythrocytosis can be caused by mutations in genes involved in the hypoxia-inducible factor (HIF) pathway.[1-3] For example, Chuvash polycythemia is caused by an R200W substitution in the von Hippel–Lindau protein (VHL).[1] There is increasing evidence linking VHL-HIF dysregulation to altered vascular physiology, and a mouse model of Chuvash polycythemia develops pulmonary arterial hypertension (PAH).[4-6] Recently, we reported an autosomal dominant erythrocytosis associated with an activating *EPAS-1* (*HIF-2A*) mutation in which there was late-onset PAH in some family members.[7] We now report a patient with severe erythropoietic dysregulation and PAH who is a compound heterozygote for novel *VHL* mutations.

A 2-month-old boy presented with increasing dyspnea and hypoxia requiring emergency ventilation and inotropic support. Echocardiography showed right ventricular dysfunction and hypertrophy. Severe PAH was confirmed by cardiac catheterization. Pulmonary artery systolic pressure was 91 mm Hg (approximately twice systemic values). Infusions of nitric oxide, prostacyclin, and sildenafil were required to allow discontinuation of ventilation. Treatment with vasodilators, diuretics, and bosentan was continued on eventual discharge from hospital.

Consistently raised hemoglobin (Hb) concentrations (> 21 g/dL) prompted further investigation. Serum erythropoietin (EPO) concentration was grossly elevated at 4120 IU/L. Diagnostic imaging and selective venous sampling provided no evidence of an EPO-secreting lesion. We hypothesized that this unusual phenotype was explicable by congenital dysregulation of the HIF pathway. Gene sequencing revealed heterozygous mutations in exon 2 (376 G>A) and exon 3 (548 C>T) of *VHL* (Figure 1A), predicting the amino acid changes Asp126Asn (D126N) and Ser183Leu (S183L), respectively.

To examine the functional consequences of the mutations, VHL-null renal carcinoma cells were transfected to generate cell pools stably expressing wild type (WT) or mutant proteins (Figure 1C). Function was assessed by measurement of the pH of cell culture media. Impaired or absent VHL function results in more rapid acidification because of HIF-mediated enhancement of glycolysis and suppression of mitochondrial respiration.[8,9] As expected, expression of WT VHL increased media pH while an inactivating VHL mutation (N78S) had no effect. In contrast, each of the D126N and S183L mutants exhibited an intermediate effect (Figure 1B). Pools expressing mutant proteins consumed more glucose and produced more lactate compared with WT, consistent with enhanced glycolytic metabolism (Figure 1B). To confirm that D126N and S183L mutations impair the ability of VHL to regulate HIF, we examined HIF-1α protein levels (Figure 1C) and the expression of HIF target genes *PHD3* and *GLUT-1* (Figure 1D), all of which were elevated in comparison to WT.

Thus, our patient has compound heterozygosity for novel mutations in VHL, which impair the ability to regulate HIF. Strikingly, EPO levels are greatly in excess of those observed in previous patients with inherited VHL-HIF dysfunction, suggesting that this patient has a more severe defect in HIF regulation. We observed that D126N and S183L were expressed at lower levels in transfected cells compared with WT. Because stably transfected cell pools exhibit a range of expression of the introduced protein, we examined this in multiple clonal sublines, with similar results. We hypothesized that this could reflect intrinsic differences in the

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ORIGINAL ARTICLE

Use of mycophenolate mofetil in patients received allogeneic hematopoietic stem cell transplantation in Japan

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Abstract We evaluated the use of mycophenolate mofetil (MMF) after hematopoietic stem cell transplantation (HSCT) in Japan from 1999 to 2008. MMF was administered to 301 patients, including 157 for the prevention of graft-versus-host disease (GVHD), 94 for the treatment of acute GVHD and 50 for the treatment of chronic GVHD. The three most common doses were 500 mg twice daily, 250 mg three times daily and 1,000 mg twice daily, given to 63, 54 and 45 patients, respectively. The incidence of grade II–IV acute GVHD was 30.0% and grade III–IV was 20.0% in the GVHD prevention group. Among treated patients, disappearance or improvement of subjective symptoms occurred in 57.0% of acute GVHD patients and in 52.0% of chronic GVHD patients. With regard to safety, the following major adverse events (grade 3 or more) were recorded: 31 infections, 31 neutropenia, 28 thrombocytopenia, 25 diarrhea and 1 renal disorder. A total of 116 patients developed grade 3 or 4 adverse events, but 79 were successfully treated with supportive treatment. Thus, our findings suggest that MMF is safe and effective for the

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prevention and treatment of GVHD in patients who have received an allogeneic stem cell transplant.

Keywords Mycophenolate mofetil (MMF) · Allogeneic stem cell transplantation · GVHD

1 Introduction

Acute and chronic graft-versus-host disease (GVHD) are important complications following allogeneic hematopoietic stem cell transplantation (HSCT) that can be prevented or treated by immunosuppressive agents such as cyclosporine, tacrolimus, steroids or other therapies [1–3]. Some patients, however, do not respond to these conventional treatments. It is well recognized that mycophenolate mofetil (MMF) is widely used in countries outside Japan, and numerous reports have documented its efficacy for prophylaxis and treatment of GVHD [4–13].

In Japan, MMF is only approved as an immunosuppressant drug for organ transplantation (e.g., renal transplantation) and has not been approved for prophylactic or therapeutic use for GVHD in the field of HSCT. As there have been several reports of experimental MMF use for HSCT in Japan [14, 15], we conducted a nationwide survey to determine the efficacy and safety of MMF in the Japanese population.

2 Patients and methods

2.1 Study design

We retrospectively collected data on MMF use after allogeneic HSCT from related donors. Questionnaires were sent to 228 institutes registered with the Japan Society for Hematopoietic Cell Transplantation (JSHCT). A total of 57 surveys were returned detailing 301 patients undergoing MMF treatment. Data regarding the purpose of treatment, dosage, length of treatment, presence or absence of subjective symptoms of GVHD, GVHD grade and stage (before and after treatment), decrease or increase in concomitant immunosuppressants, effects, adverse events and outcomes were collected. Basic information for each transplantation was extracted from the Transplant Registry Unified Management Program (TRUMP) system, which is a registry used for Japanese patient outcomes [16]. Several demographic data were not available due to the lack of patient entry into the TRUMP system. The effects of MMF with regard to subjective symptoms (none, disappearance, improvement, no change and ingravescence) and the use of steroids (none, withdrawal, dose reduction, no change and dose increase) were assessed by physicians. Adverse events were evaluated by the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE, ver.3). This study was approved by the ethical committees of the Japan Society of Hematopoietic Cell Transplantation and the Nagoya University School of Medicine.

2.2 Statistics

Correlations between the two subgroups were examined using the χ^2 test and Fisher's exact test. P values of less than 0.05 obtained in two-sided tests were considered statistically significant. The data were analyzed with STATA version 10 statistical software (STATA Corp, TX).

3 Results

3.1 Patient background

Patient background data are summarized in Table 1. Patient age ranged from 12 to 70 years (median 41) at the time of transplantation, and there were 173 (57.5%) male and 128 (42.5%) female patients. Among the 301 patients, 97 (32.2%) received a transplant from HLA-matched donor, and 182 (60.5%), from HLA-mismatched donors. Of the HLA-mismatched donors, 66 (36.3%) were 1 locus, 46 (25.3%) were 2 loci and 55 (30.2%) were 3 loci mismatched. There were also 22 patients (7.3%) with missing HLA data. Among the 157 patients who received MMF for GVHD prophylaxis, 119 (75.8%) received a transplant from an HLA-mismatched donor, and among the 50 patients who received MMF as a treatment for chronic GVHD, 17 (34.0%) received a transplant from an HLA-mismatched donor. The graft source was peripheral blood stem cells (PBSCs) in 176 patients, bone marrow (BM) in 101 patients and PBSCs plus BM in 2 patients. The preconditioning regimen was myeloablative in 91 patients and non-myeloablative in 166 patients. Table 1 shows that the primary disease was hematological malignancy in the majority of patients (94.4%) with aplastic anemia or other diseases accounting for the remainder of the patients. Among the patients with the hematological malignancies, 65.9% (162/246, which is clear data of disease status) were in non-complete remission at the time of transplantation.

3.2 MMF administration

The aim of MMF administration was GVHD prevention in 157 patients, acute GVHD treatment in 94 patients and chronic GVHD treatment in 50 patients (Table 1). The daily MMF dosage varied from 250 to 3,000 mg, and the number of doses per day ranged from 1 to 8. The most common dosages and frequencies of MMF administration were

Table 1 Patient characteristics

Variables	Number
Patient number	301
Median age (range)	41 (12–70)
Male/female	173/128
Disease[a]	
Acute myeloid leukemia	78 (46)
Acute lymphoblastic leukemia	66 (44)
Chronic myelogenous leukemia	15 (11)
Myelodysplastic/myeloproliferative syndrome	39 (12)
Malignant lymphoma	75 (41)
Multiple myeloma	11 (8)
Aplastic anemia	3
Other diseases	14 (11)
Purpose of MMF	
GVHD prophylaxis	157
aGVHD treatment	94
cGVHD treatment	50
Graft source[b]	
Bone marrow (BM)	101
Peripheral blood stem cell (PBSC)	176
Both BM and PBSC	2
Donor type[b]	
Matched related	97
Mismatched related	182
1 locus mismatch	66
2 loci mismatch	46
3 loci mismatch	55
Unknown	15

[a] Numbers in parenthesis indicate those of not in complete remission

[b] Twenty-two data were missing for graft source and donor type

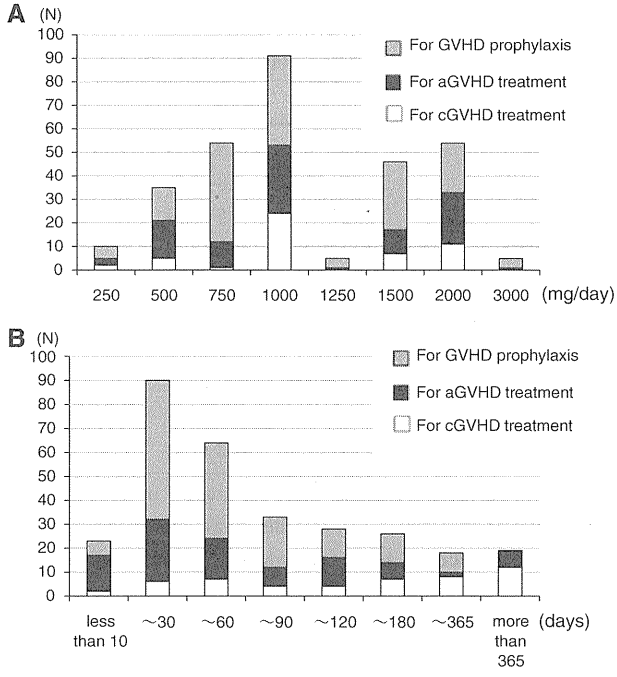

Fig. 1 **a** Initial dose of MMF. MMF was given at a variety of doses ranging from 250 mg per day to 3,000 mg per day. The most common dose was 500 mg twice a day ($N = 67$ among 91 patients taking 1,000 mg per day). **b** Dosing period of MMF. MMF was given for a variety of dosing periods (median 45 days)

500 mg two times per day, 250 mg three times per day and 1,000 mg two times per day given to 63 patients (20.9%), 54 patients (17.9%) and 45 patients (15.0%), respectively. Consequently, 91 patients received 1,000 mg of MMF per day, and 54 patients, 750 or 2,000 mg per day. 59 patients were treated with a daily dose higher than 2,000 mg. There was no consistent pattern between the length and purpose of treatment. MMF administration was discontinued within 30 days in 113 patients (38.4%); however, 19 patients received MMF for more than a year (Fig. 1). Most patients (289 patients, 96.0%) were given MMF concurrently with other immunosuppressants (e.g., cyclosporine, tacrolimus or steroids), and only 12 patients (4%) received MMF alone.

3.3 Adverse events

Adverse events (AEs) associated with MMF administration are listed in Table 2. The major events were neutropenia, infection, thrombocytopenia and myelosuppression. Only three patients (1.7%) developed renal insufficiency with a grade 1, 2 or 4 increase in creatinine. Eighteen patients (6.0%) died from AEs associated with MMF (Table 3). The primary causes of death were infections in 11 patients (including 5 patients with pneumonia, 4 with sepsis and 2 with invasive *Aspergillus* infection), neutropenia in 3 patients, myelosuppression in 2 patients, 1 thrombocytopenia and 1 brain hemorrhage. There were 44 grade 4 AEs: 25 of these patients (56.8%) improved and 15 (34.1%) remained unchanged, but 4 (9.1%) eventually died. The incidence of AEs of grade 3 or higher (except infection) increased in accordance with the daily dosage of MMF (Fig. 2), but most of these AEs improved (Table 4).

3.4 Efficacy of MMF

Among the 157 patients who received MMF for GVHD prophylaxis, the incidences of grade II–IV and grade III–IV acute GVHD were 29.7% (43/145) and 20.0% (29/145), respectively. Limited and extensive chronic GVHD occurred in 21 (18.6%) and 30 (26.6%) patients, respectively ($N = 113$). No significant differences were found in the incidence of grade II–IV acute GVHD between HLA-matched and mismatched transplant patients ($9/25 = 36.0$ vs. $33/113 = 29.2\%$, $P = 0.63$), and no significant differences were noted between these two groups with regard to the

Table 2 Adverse events whose relationships to MMF were not necessarily denied

Adverse events: all (grade 3–5)	GVHD prophylaxis ($N = 157$)		aGVHD treatment ($N = 94$)		cGVHD treatment ($N = 50$)		Total ($N = 301$)	
	N	%	*N*	%	*N*	%	*N*	%
Infection	6 (5)	3.8 (3.2)	16 (13)	17.0 (13.8)	9 (8)	18.0 (16.0)	31 (26)	10.3 (8.6)
Diarrhea	6 (5)	3.8 (3.2)	16 (10)	17.0 (10.6)	3 (3)	6.0 (6.0)	25 (18)	8.3 (6.0)
Nausea	7 (2)	4.5 (1.3)	6 (4)	6.4 (4.3)	3 (0)	6.0 (0)	16 (6)	5.3 (2.0)
Vomiting	2 (0)	1.3 (0)	2 (0)	2.1 (0)	1 (0)	2.0 (0)	5 (0)	1.7 (0)
Neutropenia	5 (5)	3.2 (3.2)	21 (20)	22.3 (21.3)	5 (5)	10.0 (10.0)	31 (30)	10.3 (10.0)
Thrombocytopenia	5 (5)	3.2 (3.2)	18 (15)	19.1 (16.0)	5 (5)	10.0 (10.0)	28 (25)	9.3 (8.3)
Myelosuppression	7 (7)	4.5 (4.5)	10 (7)	10.6 (7.4)	4 (4)	8.0 (8.0)	21 (18)	7.0 (6.0)
Gastrointestinal bleeding	3 (2)	1.9 (1.3)	3 (3)	3.2 (3.2)	0 (0)	0 (0)	6 (5)	20.0 (1.7)
Constipation	1 (0)	0.6 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0.3 (0)
Others	5 (3)	3.2 (1.9)	7 (3)	7.4 (3.2)	3 (2)	6.0 (4.0)	15[a] (8[b])	5.0 (2.7)

Numbers in parenthesis indicate those for grade 3 or more toxicity

[a] Others: liver dysfunction (3), creatine kinase elevation (2), hair loss, hemorrhage cystitis

[b] Others: hypocalcemia, brain hemorrhage, septic shock, creatine kinase elevation, abdominal pain, TMA, diabetes mellitus, engraft failure

Table 3 Cause of death potentially associated with MMF

	Number
Infection	11
Pneumonia	5
Bacterial	2
MRSA	1
Fungal	1
CMV	1
Sepsis	4
Invasive *Aspergillus* infection	2
Neutropenia	3
Myelosuppression	2
Thrombocytopenia	1
Brain hemorrhage	1
Total	18

incidence of grade III–IV acute GVHD (6/25 = 24.0 vs. 22/113 = 19.5%, $P = 0.59$). The incidence of chronic GVHD, however, tended to be lower in the HLA-mismatched transplant group (14/23 = 60.9 vs. 35/83 = 42.2%, $P = 0.16$; Fig. 3), although this finding was not statistically significant. The incidences of grade II–IV and III–IV acute GVHD were lower in the subgroup of patients receiving 2,000 mg of MMF daily than in the subgroup receiving 1,000 mg daily (28.6 vs. 37% and 14.3 vs. 28.6% for grade II–IV and III–IV acute GVHD, respectively), although these differences were not statistically significant ($P = 0.51$ and 0.22, respectively). No dose effect was found for chronic GVHD prevention ($P = 0.72$).

Among the 94 patients in the acute GVHD treatment group, subjective symptoms disappeared in 27 (28.7%) and improved in 28 (29.8%). Symptoms remained unchanged in 17 patients (18.1%) and worsened in 22 patients (23.4%). Within this treatment group, 52 patients (55.3%) experienced improvement in their acute GVHD grade. Treatment with combined immunosuppressants was discontinued in 5 patients (5.3%) and reduced in 51 patients (54.3%). Among the 50 patients who received MMF as a treatment for chronic GVHD, the drug was effective against subjective symptoms (i.e., resulted in resolution or improvement) in 52.0% (10.0 and 42.0% experiencing resolution and improvement, respectively). Five patients (10.0%) discontinued combined immunosuppressants, and 29 (58.0%) reduced their dosage. The dosage remained unchanged in 14 patients (28.0%) and increased in only 2 patients (4%) (Fig. 4). In the acute GVHD treatment group, the effectiveness of MMF was higher among patients who had received HLA-matched transplants; however, this difference was not statistically significant for all items evaluated (58–70 vs. 32–69%, $P = 0.18$–0.60). In the chronic GVHD treatment group, the efficacy of MMF against subjective symptoms was higher in the HLA-matched subgroup than in the HLA-mismatched subgroup (17/33 = 51.5 vs. 3/9 = 33.3%, respectively, $P = 0.45$). In contrast, the rate of dosage reduction or discontinuation for combined immunosuppressants was higher in the HLA-mismatched subgroup than in the HLA-matched subgroup (7/9 = 77.8 vs. 21/33 = 63.6%, respectively, $P = 0.69$).

To assess the efficacy of MMF with regard to total daily dosage, we selected two subgroups: the most frequent dosage (1,000 mg per day) and the maximum dosage (more than 2,000 mg per day). The efficacy rate for every acute GVHD survey item was virtually identical between the 1,000 mg per day ($N = 28$) and 2,000 mg per day ($N = 23$) subgroups

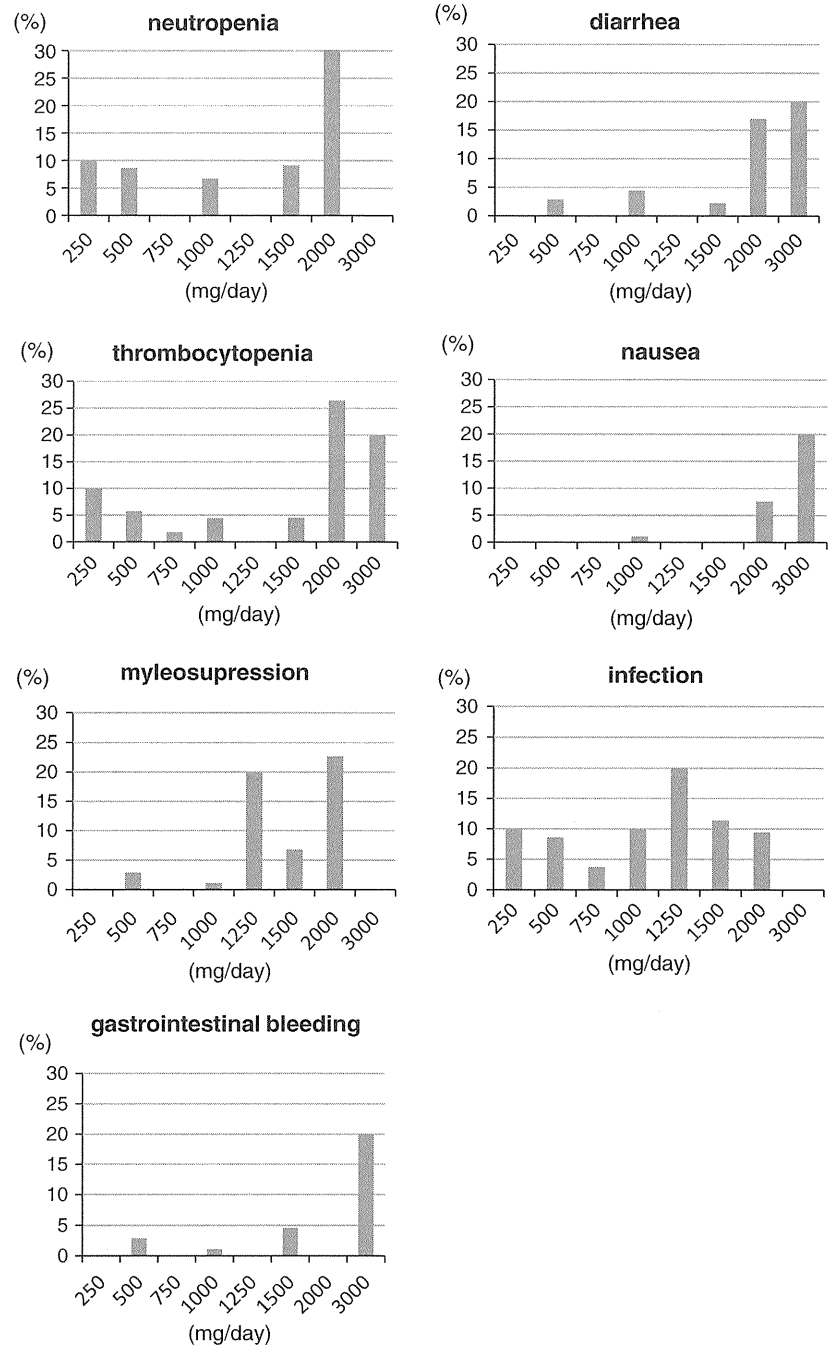

Fig. 2 Frequency of adverse events (grades 3–5) separated by total daily dose. High doses of MMF resulted in higher rates of hematological and gastrointestinal adverse events. Infections developed at all doses of MMF

(47.8–70.8 vs. 33.3–72.7%, respectively, $P = 0.06$-0.97). Among chronic GVHD patients, no difference in dose efficacy was observed between the two dosage subgroups ($N = 24$ in the 1,000 mg per day group and $N = 11$ for patients taking more than 2,000 mg per day, $P = 0.83$–0.91).

3.5 Transplantation outcome

In the GVHD prevention group, engraftment was seen in 122 of 134 evaluable patients (91.0%). Among all 301 patients, 62 (20.7%) relapsed and 169 (56.2%) died after transplantation. The overall survival rate was 41.9% at a median follow-up of 3 years. The main causes of death included disease recurrence in 33 patients (responsible for 19.5% of patient mortality), infection in 26 patients (15.4%), acute GVHD in 26 patients (15.4%) and chronic GVHD in 7 patients (4.1%). Among the 26 deaths due to acute GVHD, 18 patients were in the acute GVHD treatment group. Among the seven patients who died due to chronic GVHD, four were in the chronic GVHD treatment group.

Table 4 Rate of recovery from the adverse events in grades 3–4

	1,000 mg/day ($N = 91$)	More than 2,000 mg/day ($N = 59$)	Total ($N = 301$)
Infection	1/4 (25%)	1/2 (50%)	12/16 (75%)
Diarrhea	3/4 (75%)	7/10 (70%)	10/16 (63%)
Nausea	0/1 (0%)	4/5 (80%)	4/6 (67%)
Neutropenia	6/6 (100%)	12/15 (80%)	24/27 (89%)
Thrombocytopenia	3/4 (75%)	5/14 (36%)	11/24 (46%)
Myelosuppression	1/1 (100%)	8/11 (73%)	12/16 (75%)
Gastrointestinal bleeding	1/1 (100%)	0/1 (0%)	2/5 (40%)

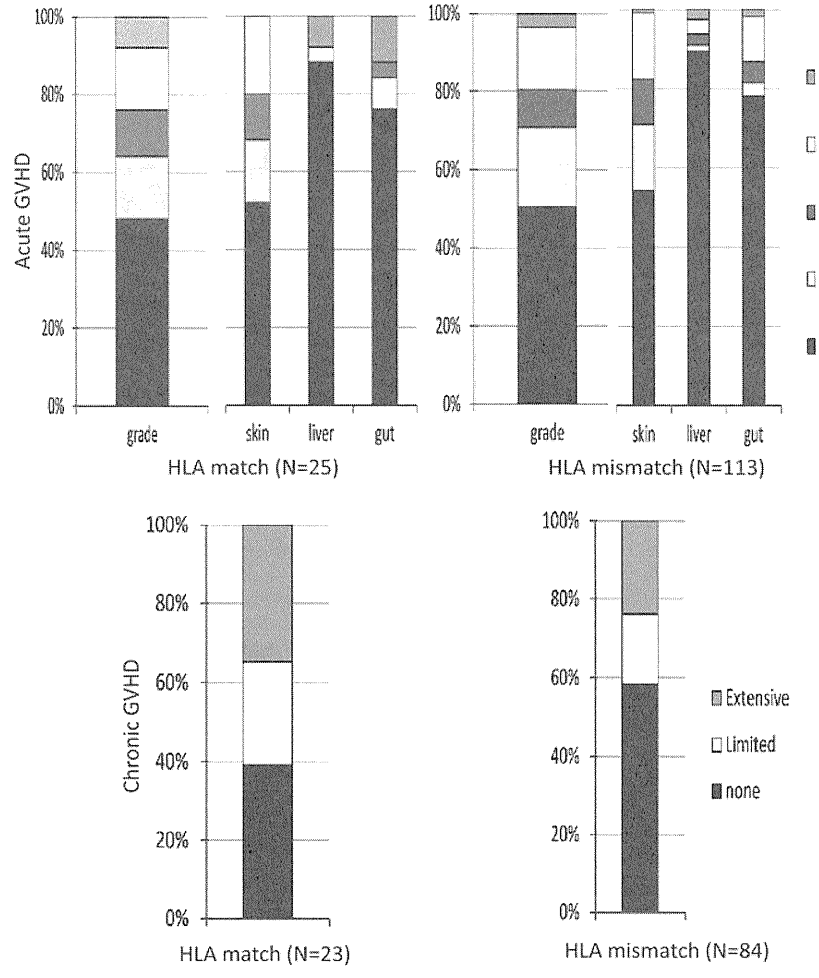

Fig. 3 Incidence of GVHD with prophylactic MMF use. The incidences of grade II–IV acute GVHD were 36.0 and 29.2% in the HLA-matched and -mismatched subgroups, respectively. In contrast, the incidence of chronic GVHD in the HLA-mismatched subgroup was lower (42.2%) than in the HLA-matched subgroup (60.9%)

4 Discussion

GVHD is one of the leading complications following allogeneic HSCT and is associated with morbidity and mortality. Calcineurin inhibitors and steroids are widely used for GVHD prevention and treatment, but several other immunosuppressive agents have been used for these purposes overseas [17–19]. Since 1997, many promising reports have compared MMF with conventional immunosuppressants [4–13]. In particular, reports focused on GVHD prevention are becoming increasing common due to the use of alternative donor sources [20]. Our current survey demonstrates that the efficacy rate of MMF is approximately 60% for the treatment of acute and chronic GVHD. Furthermore, our results also reveal that MMF is effective for the prevention of GVHD. Especially in HLA-mismatched patients, the frequency of grade III–IV acute GVHD was 20.3%, which was lower than the previous report subjected to HLA-mismatched transplants among Japanese populations [21]. As the efficacy of MMF was

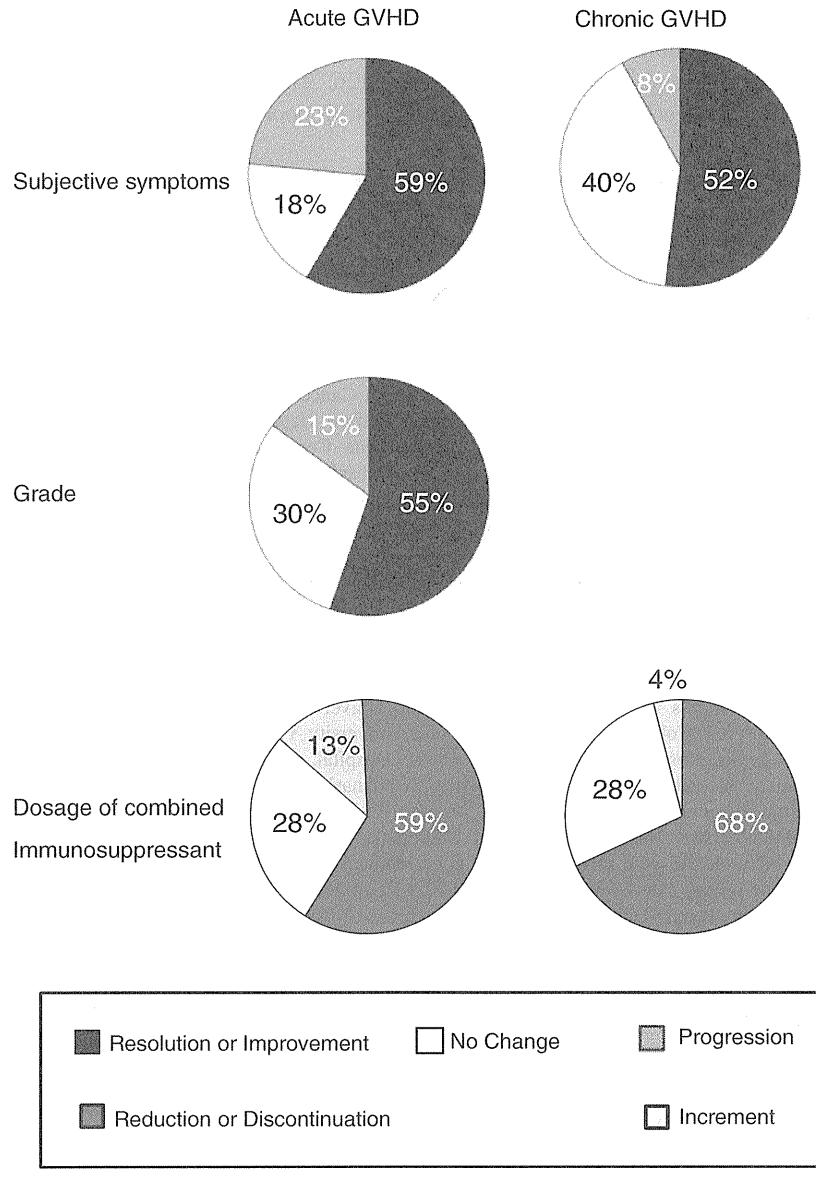

Fig. 4 Response of acute and chronic GVHD during therapeutic MMF use. Subjective symptoms of acute and chronic GVHD resolved in 59 and 52% of the cases, respectively, following the administration of MMF. In addition, 55% of the acute GVHD patients improved the grade of their disease. Finally, 60 and 68% of the acute and chronic GVHD patients, respectively, reduced or discontinued their use of combined immunosuppressant therapy

higher in patients receiving 2,000 mg per day than in those receiving 1,000 mg per day for chronic GVHD prevention, MMF doses of more than 2,000 mg per day are recommended for Japanese patients if the AEs are manageable.

Whether MMF is superior to existing immunosuppressants is a topic of continuing debate. Most previous reports on MMF have been promising, and the response rates for acute and chronic GVHD range from 47 to 71 and 26 to 76.9%, respectively, under various conditions [4, 6, 9–11, 17, 20]. On the other hand, one report suggested that MMF causes no significant improvement in the prevention of GVHD compared to cyclosporine and methotrexate (62 vs. 70%) [12]. Furthermore, another report showed that addition of MMF to an immunosuppressive regimen to control chronic GVHD had no effect (success rate of 15%) [22]. The results in this survey are not statistically different between using MMF and using cyclosporine or tacrolimus as reported in the previous report for the prevention and treatment of GVHD. We would like to emphasize, however, that the patient population in this study consisted mostly of HLA-mismatched donors and non-complete remission recipients (60.5 and 65.9%, respectively; Table 1). Even in this situation, MMF showed comparable efficacy. Therefore, we would like to conclude that MMF has a certain role for immunosuppressants.

Several reports have noted that the incidence of renal damage attributed to MMF (0–12.5%) is lower than that reported for other immunosuppressants like calcineurin inhibitors [4, 5, 11, 12, 23–25]. Our analysis revealed that the incidence of renal insufficiency (serum creatinine > 2 mg/dl)

was 1%. Serum creatinine > 2 mg/dl due to treatment with calcineurin inhibitors can be as high as 50–60 and 56–67% for cyclosporine and tacrolimus, respectively [26, 27]. Thus, MMF will be especially useful for patients with poor renal function.

In conclusion, MMF is tolerable and effective in Japanese patients who have received HSCT. Further studies are warranted to identify suitable candidates and appropriate therapeutic combinations of MMF for the prophylaxis and treatment of GVHD following allogeneic HSCT.

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References

1. Goker H, Haznedaroglu IC, Chao NJ. Acute graft-vs-host disease: pathobiology and management. Exp Hematol. 2001;29:259–77.
2. Martin PJ, Schoch G, Fisher L, Byers V, Anasetti C, Appelbaum FR, et al. A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. Blood. 1990;76:1464–72.
3. Martin PJ, Carpenter PA, Sanders JE, Flowers ME. Diagnosis and clinical management of chronic graft-versus-host disease. Int J Hematol. 2004;79:221–8.
4. Basara N, Blau WI, Kiehl MG, Romer E, Rudolphi M, Bischoff M, et al. Efficacy and safety of mycophenolate mofetil for the treatment of acute and chronic GVHD in bone marrow transplant recipient. Transplant Proc. 1998;30:4087–9.
5. Basara N, Blau WI, Kiehl MG, Schmetzer B, Bischoff M, Kirsten D, et al. Mycophenolate mofetil for the prophylaxis of acute GVHD in HLA-mismatched bone marrow transplant patients. Clin Transplant. 2000;14:121–6.
6. Basara N, Blau WI, Romer E, Rudolphi M, Bischoff M, Kirsten D, et al. Mycophenolate mofetil for the treatment of acute and chronic GVHD in bone marrow transplant patients. Bone Marrow Transplant. 1998;22:61–5.
7. Bolwell B, Sobecks R, Pohlman B, Andresen S, Rybicki L, Kuczkowski E, et al. A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. Bone Marrow Transplant. 2004;34:621–5.
8. Bornhauser M, Schuler U, Porksen G, Naumann R, Geissler G, Thiede C, et al. Mycophenolate mofetil and cyclosporine as graft-versus-host disease prophylaxis after allogeneic blood stem cell transplantation. Transplantation. 1999;67:499–504.
9. Busca A, Saroglia EM, Lanino E, Manfredini L, Uderzo C, Nicolini B, et al. Mycophenolate mofetil (MMF) as therapy for refractory chronic GVHD (cGVHD) in children receiving bone marrow transplantation. Bone Marrow Transplant. 2000;25:1067–71.
10. Kim JG, Sohn SK, Kim DH, Lee NY, Suh JS, Lee KS, et al. Different efficacy of mycophenolate mofetil as salvage treatment for acute and chronic GVHD after allogeneic stem cell transplant. Eur J Haematol. 2004;73:56–61.
11. Mookerjee B, Altomonte V, Vogelsang G. Salvage therapy for refractory chronic graft-versus-host disease with mycophenolate mofetil and tacrolimus. Bone Marrow Transplant. 1999;24:517–20.
12. Nash RA, Johnston L, Parker P, McCune JS, Storer B, Slattery JT, et al. A phase I/II study of mycophenolate mofetil in combination with cyclosporine for prophylaxis of acute graft-versus-host disease after myeloablative conditioning and allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2005;11:495–505.
13. Neumann F, Graef T, Tapprich C, Vaupel M, Steidl U, Germing U, et al. Cyclosporine A and mycophenolate mofetil vs cyclosporine A and methotrexate for graft-versus-host disease prophylaxis after stem cell transplantation from HLA-identical siblings. Bone Marrow Transplant. 2005;35:1089–93.
14. Okamura A, Yamamori M, Shimoyama M, Kawano Y, Kawano H, Kawamori Y, et al. Pharmacokinetics-based optimal dose-exploration of mycophenolate mofetil in allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2008;88:104–10.
15. Takami A, Mochizuki K, Okumura H, Ito S, Suga Y, Yamazaki H, et al. Mycophenolate mofetil is effective and well tolerated in the treatment of refractory acute and chronic graft-versus-host disease. Int J Hematol. 2006;83:80–5.
16. Atsuta Y, Suzuki R, Yoshimi A, Gondo H, Tanaka J, Hiraoka A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. Int J Hematol. 2007;86:269–74.
17. Alousi AM, Weisdorf DJ, Logan BR, Bolanos-Meade J, Carter S, Difronzo N, et al. Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network. Blood. 2009;114:511–7.
18. Lee SJ, Vogelsang G, Gilman A, Weisdorf DJ, Pavletic S, Antin JH, et al. A survey of diagnosis, management, and grading of chronic GVHD. Biol Blood Marrow Transplant. 2002;8:32–9.
19. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2003;9:215–33.
20. Furlong T, Martin P, Flowers ME, Carnevale-Schianca F, Yatscoff R, Chauncey T, et al. Therapy with mycophenolate

mofetil for refractory acute and chronic GVHD. Bone Marrow Transplant. 2009;44:739–48.
21. Kanda Y, Chiba S, Hirai H, Sakamaki H, Iseki T, Kodera Y, et al. Allogeneic hematopoietic stem cell transplantation from family members other than HLA-identical siblings over the last decade (1991–2000). Blood. 2003;102:1541–7.
22. Martin PJ, Storer BE, Rowley SD, Flowers ME, Lee SJ, Carpenter PA, et al. Evaluation of mycophenolate mofetil for initial treatment of chronic graft-versus-host disease. Blood. 2009;113:5074–82.
23. Arai S, Vogelsang GB. Management of graft-versus-host disease. Blood Rev. 2000;14:190–204.
24. Bornhauser M, Thiede C, Schuler U, Platzbecker U, Freiberg-Richter J, Helwig A, et al. Dose-reduced conditioning for allogeneic blood stem cell transplantation: durable engraftment without antithymocyte globulin. Bone Marrow Transplant. 2000;26:119–25.
25. Krejci M, Doubek M, Buchler T, Brychtova Y, Vorlicek J, Mayer J. Mycophenolate mofetil for the treatment of acute and chronic steroid-refractory graft-versus-host disease. Ann Hematol. 2005;84:681–5.
26. Nash RA, Antin JH, Karanes C, Fay JW, Avalos BR, Yeager AM, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000;96:2062–8.
27. Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998;92:2303–14.

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Acceptable HLA-mismatching in unrelated donor bone marrow transplantation for patients with acquired severe aplastic anemia

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Acceptable HLA-mismatching in unrelated donor bone marrow transplantation for patients with acquired severe aplastic anemia

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We retrospectively analyzed the effect of HLA mismatching (HLA-A, -B, -C, -DRB1, -DQB1) with molecular typing on transplantation outcome for 301 patients with acquired severe aplastic anemia (SAA) who received an unrelated BM transplant through the Japan Marrow Donor Program. Additional effect of HLA-DPB1 mismatching was analyzed for 10 of 10 or 9 of 10 HLA allele-matched pairs (n = 169). Of the 301 recipient/donor pairs, 101 (33.6%) were completely matched at 10 of 10 alleles, 69 (23%) were mismatched at 1 allele, and 131 (43.5%) were mismatched at ≥ 2 alleles. Subjects were classified into 5 subgroups: complete match group (group I); single-allele mismatch group (groups II and III); multiple alleles restricted to HLA-C, -DRB1, and -DQB1 mismatch group (group IV); and others (group V). Multivariate analysis indicated that only HLA disparity of group V was a significant risk factor for poor survival and grade II-IV acute GVHD. HLA-DPB1 mismatching was not associated with any clinical outcome. We recommend the use of an HLA 10 of 10 allele-matched unrelated donor. However, if such a donor is not available, any single-allele or multiple-allele (HLA-C, -DRB1, -DQB1) mismatched donor is acceptable as an unrelated donor for patients with severe aplastic anemia. (*Blood.* 2011;118(11):3186-3190)

Introduction

BM transplantation from an unrelated donor (UBMT) is indicated as salvage therapy for patients with severe aplastic anemia (SAA) who fail to respond to immunosuppressive therapy. Early results of UBMT have not been encouraging because of a high incidence of graft failure and GVHD.[1-3] The Center for International Blood and Marrow Transplant Research (CIBMTR) reported the outcome of 232 patients with SAA who received an UBM transplant between 1988 and 1998.[3] The 5-year probabilities of overall survival (OS) were 39% and 36% after matched unrelated and mismatched unrelated donor transplantations, respectively. We previously reported the outcome of 154 patients with SAA who received an UBM transplant between 1993 and 2000 through the Japan Marrow Donor Program (JMDP).[4] The 5-year OS rate was 56% in that study.

In several recent studies, the effect of HLA high-resolution matching on outcome of patients who received an UBM transplant has been elucidated.[5-8] However, results have been derived primarily from an analysis of patients with hematologic malignancies. Major obstacles for UBMT are different between patients with hematologic malignancies and patients with SAA. Relapse is a main cause of death for patients with hematologic malignancies, and GVL effect may result in decrease of relapse rate. In contrast, graft failure is the main problem, and GVHD is the only negative effect for patients with SAA. Therefore, optimal HLA matching may be different between these 2 populations. Algorithms for donor selection derived from an analysis of patients with hematologic malignancies might not be useful for patients with SAA. However, a few studies have focused on the clinical significance of HLA-allele compatibility in patients with SAA.[2,4,9,10]

In a previous study, we analyzed the clinical significance of HLA allele mismatching in 142 patients with SAA, in whom data of high-resolution typing of HLA-A, -B, and -DRB1 were available.[4] Mismatching of HLA-A or -B alleles between donor and recipient was a strong risk factor for acute and chronic GVHD and OS, whereas mismatching of the HLA-DRB1 allele did not have a significant effect on patient outcomes. In the study from the National Marrow Donor Program, mismatching of HLA-DRB1 was the most crucial risk factor for OS.[2] These results indicate that better donor selection through high-resolution typing might result in improved outcome in patients with SAA who receive an UBM transplant. In fact, several recent studies showed a significantly improved outcome in patients with SAA who received and UBM transplant over time.[11,12] In particular, better HLA matching by high-resolution typing has been thought to contribute to these improvements.[4,9-11]

On the contrary, restricting BMT to donor-recipient pairs perfectly matched at high-resolution typing reduces the chance of undergoing UBMT for many patients. Therefore, strategies for selecting a partially HLA allele mismatched donor are required when a full matched donor cannot be identified. Here, we report a detailed analysis of outcome in 301 patients with SAA who were

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typed for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 by a molecular technique and underwent UBMT through the JMDP.

Methods

Patients

From February 1993 to April 2005, 380 consecutive patients with acquired SAA received an UBM transplant through the JMDP. Patients with inherited AA, such as Fanconi anemia, and patients who received a BM transplant > 2 times were excluded. This study includes 301 patients in whom molecular analysis of HLA-A, -B, -C, DRB1, and -DQB1 were performed by DNA-based methods. HLA-DPB1 was analyzed in 299 of these patients. The previous study included 142 patients in whom molecular typing was performed only for HLA-A, -B, and -DRB1.

Characteristics of the 301 patients and donors are shown in Table 1. Briefly, patients (173 males and 128 females) were between birth and 64 years of age (median, 17 years of age). The median disease duration before BMT was 43 months (range, 4-436 months). All patients failed conventional immunosuppressive therapies and were considered candidates for UBMT. All patients or their guardians gave informed consent for transplantation and submission of the data to the JMDP.

Transplantation procedure

Characteristics of the transplantation procedures are also shown in Table 1. Patients underwent transplantations at individual centers following the local protocols for preconditioning regimens and GVHD prophylaxis. The various preconditioning regimens used by individual centers were classified into 5 categories: TBI or LFI + CY + ATG (n = 128), TBI or LFI + CY (n = 103), TBI or LFI + CY + Flu with or without ATG (n = 39), CY + Flu + ATG (n = 8), and others (n = 23). In 130 patients, CsA and MTX were used for prophylaxis against GVHD; 134 patients received FK instead of CsA. The remaining 35 patients received other GVHD prophylaxis. Ex vivo T-cell depletion was not used for any patient. The median number of infused nucleated marrow cells was 3.1×10^8/kg. One-half (n = 150) of the transplantations were performed before 2000, and 151 were done after 2001.

HLA typing and definition of mismatching

HLA matching between patients and donors was based on HLA serotyping according to the standard technique. Partial HLA-A and -B alleles and complete HLA-DRB1 alleles were identified as confirmatory HLA typing during the coordination process, and HLA-A, -B, -C, -DQB1, and -DPB1 alleles were retrospectively reconfirmed or identified after transplantation. Molecular typing of HLA-A, -B, -C, -DQB1, -DRB1, and -DPB1 alleles was performed by the Luminex microbead method (Luminex 100 system) adjusted for the JMDP and in part by the sequencing-based typing method. Mismatching was defined as the presence of donor antigens or alleles not shared by the recipient (rejection vector) or the presence of recipient antigens or alleles not shared by the donor (GVHD vector).

Definition of transplantation-related events

The day of engraftment was defined as the first day of 3 consecutive days on which neutrophil count exceeded 0.5×10^9/L. Patients who did not reach neutrophil counts > 0.5×10^9/L for 3 consecutive days after transplantation were considered to have primary graft failure. Patients with initial engraftment in whom absolute neutrophil counts declined to < 0.5×10^9/L subsequently were considered to have secondary graft failure. Acute GVHD was evaluated according to standard criteria in patients who achieved engraftment, and chronic GVHD was evaluated according to standard criteria in patients who achieved engraftment and survived > 100 days after transplantation.

Data collection and statistical analysis

Transplantation data were collected with the use of standardized forms provided by the JMDP. Patient baseline information and follow-up reports were submitted at 100 days and annually after transplantation. Analysis of patient outcome was performed with the date of last reported follow-up or date of death. Data were analyzed as of July 1, 2007.

Probability of OS and 95% confidence interval (95% CI) were estimated from the time of transplantation according to the Kaplan-Meier method. Cumulative incidence of neutrophil engraftment at day 42 was analyzed in the whole of patients by treating deaths until day 42 as a competing risk. Cumulative incidence of acute GVHD at day 100 was analyzed in patients who sustained engraftment by treating deaths until day 100 as a competing risk. Cumulative incidence of chronic GVHD at day 365 was analyzed in patients who sustained engraftment and survived longer than day 100 by treating deaths until day 365 as a competing risk. In univariate analysis, the log-rank test or Gray test was used to assess the significance of HLA allele mismatching on clinical outcomes. The Mann-Whitney *U* test was used to compare the median days of neutrophil engraftment. The chi-square test or Mann-Whitney *U* test was used to compare patient characteristics and transplantation procedures between the patient groups. All *P* values < .05 were considered statistically significant, whereas *P* values between .05 and .1 were considered as marginally significant.

Multivariate analyses were performed to assess the effect of HLA allele mismatching on the clinical outcome by Cox proportional hazard model (each mismatched group vs fully matched group; hazard risk = 1.0 as a reference group). Factors other than HLA mismatching included in the models were patient age, patient sex, donor age, donor sex, disease duration before BMT, infused cell dose, matching of ABO blood type, GVHD prophylaxis, and preconditioning regimens.

Results

HLA matching by DNA typing

Of the 301 recipient/donor pairs, 101 pairs (33%) were completely matched at HLA-A, -B, -C, -DRB1, and -DQB1 allele; 69 pairs (23%) were mismatched at 1 HLA allele; 59 pairs (20%) were mismatched at 2 HLA alleles; and 72 pairs (24%) were mismatched at ≥ 3 alleles (Table 2). The number and frequency of 1-allele and 2-allele mismatches in either GVHD or rejection vector or both vectors in each HLA allele were 55 (18.3%) and 7 (2.3%) in HLA-A allele, 32 (10.6%) and 2 (0.7%) in HLA-B allele, 130 (43.2%) and 10 (3.3%) in HLA-C allele, 68 (22.6%) and 5 (1.7%) in HLA-DRB1 allele, 80 (26.6%) and 13 (4.3%) in HLA-DQB1 allele, and 179 (59.5%) and 44 (14.6%) in HLA-DPB1 allele, respectively. Because the frequency of mismatching was too high at the DPB1 allele, analysis of DPB1 mismatching was separated from that of other alleles. In addition, because the number of single-allele mismatched pairs of HLA-A, -B, -C, -DRB1, and -DQB1 were too small for separate analyses, HLA-A and -B were grouped into the mismatch of the HLA-A or HLA-B allele (A/B) and HLA-DRB1 and -DQB1 into the mismatch of the HLA-DRB1 or HLA-DQB1 allele (DRB1/DQB1), respectively.

Survival

Of the 301 patients, 202 are alive at the time of analysis with an observation time from 3 to 128 months (median, 44 months) after transplantation. Five-year OS was 66.3% (95% CI, 60.7%-72.5%) in the whole population (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Subgroup analyses were performed in 8 main subgroups (> 15 recipients) as follows: (1) complete match group (n = 101), (2) single locus (A/B) mismatch group (n = 20), (3) single (C) mismatch group (n = 42), (4) 2 loci (A/B + C) mismatch group (n = 20), (5) 2 loci (DRB1/DQB1) mismatch group (n = 19), (6) 3 loci (A/B + C) mismatch group (n = 15), (7) 3 loci (C + DRB1/DQB1) mismatch group (n = 29), and

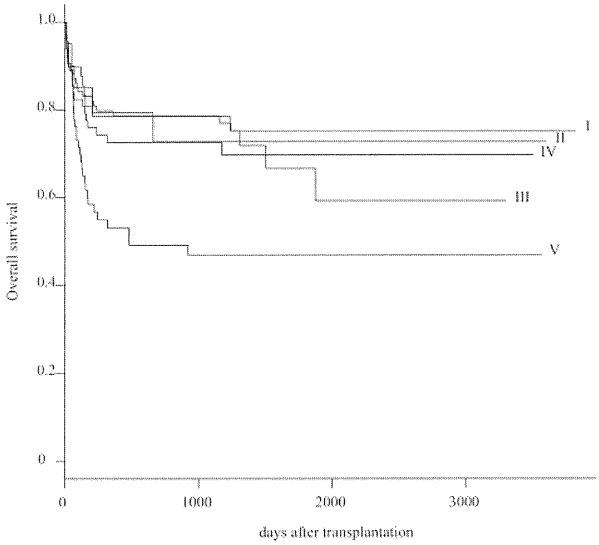

Figure 1. Kaplan-Meier estimates of OS in 5 HLA groups.

(8) 3 loci (A/B + C + DRB1/DQB1) mismatch group (n = 21). OS was significantly worse in the following groups than in the complete match group (75.2%): 2 loci (A/B + C) mismatch group (49.0%; $P = .022$), ≥ 3 loci (A/B + C) mismatch group (40.0%; $P = .002$), and A/B + C + DRB1/DQB1 mismatch group (56.1%; $P = .031$; supplemental Table 1).

On the basis of these primary results, 301 patients were reclassified into 5 subgroups: HLA complete match group (group I; n = 101), single-allele (A/B) mismatch group (group II; n = 20), single-allele (C or DRB1/DQB1) mismatch group (group III; n = 49), multiple-allele (restricted to C or DRB1/DQB1) mismatch group (group IV; n = 68), and others (group V; n = 63). The probability of OS at 5 years was 75.2% (95% CI, 84.8%-66.7%) in group I, 72.7% (95% CI, 96.7%-54.7%) in group II, 66.7% (95% CI, 85.1%-52.3%) in group III, 69.7% (95% CI, 82.6%-58.8%) in group IV, and 46.8% (95% CI, 61.7%-35.5%) in group V, respectively (Table 3; Figure 1). Survival rate was significantly inferior in group V than in group I ($P = .003$).

To avoid or minimize the effect of other HLA alleles mismatching, the effect of HLA-DPB1 mismatching was evaluated in group I (n = 101) and groups II + III (n = 69), independently. HLA-DPB1 was matched in 51 recipient/donor pairs (30%) and mismatched in 118 pairs (70%). Patient characteristics and transplantation procedures were not different between HLA-DPB1 matched and mismatched groups (supplemental Table 2). The probability of OS at 5 years in group I was equivalent between the HLA-DPB1 matched group (74.4%; 95% CI, 93.2%-59.4%) and the HLA-DPB1 mismatched group (75.7%; 95% CI, 87.2%-65.8%; $P = .894$; Table 4; Figure 2A). It was also equivalent in groups II + III (71.4%; 95% CI, 93.6%-54.5% in the HLA-DPB1 matched group and in the HLA-DPB1 mismatched group (67.1%; 95% CI, 85.6%-52.5%; $P = .826$; Table 4; Figure 2B). Multivariate analysis identified significant unfavorable variables as follows: recipient age (0-10 years: relative risk [RR] = 1.0; 11-20 years: RR = 4.092, $P = .002$; 21-40 years: RR = 3.970, $P = .004$; > 41 years: RR = 5.241, $P = .003$), conditioning regimen (Flu + CY + TBI/LFI ± ATG: RR = 1.0; CY + TBI/LFI: RR = 4.074, $P = .058$; others: RR = 6.895, $P = .013$), HLA mismatching (group I: RR = 1.0; group V: RR = 1.967, $P = .023$), donor sex (female: RR = 1.0; male: RR = 1.850, $P = .016$), and GVHD prophylaxis (FK + MTX: RR = 1.0; other: RR = 1.754, $P = .024$), blood type (ABO match or minor mismatch: RR = 1.0; major mismatch or bidirection: RR = 1.948, $P = .005$), and disease duration (< 7 years: RR = 1.0; > 7 years: RR = 1.540, $P = .084$; Table 5).

Engraftment

The cumulative incidence of neutrophil engraftment at day 42 was evaluated in 300 patients. It was 90.3% (95% CI, 93.7%-86.9%) in the whole population. Subgroup analyses showed that it was 93.0% (95% CI, 98.2%-87.8%) in group I, 90.0% (95% CI, 100%-74.6%) in group II, 89.8% (95% CI, 98.9%-80.7%) in group III, 92.6% (95% CI, 99.2%-86.0%) in group IV, and 84.1% (95% CI, 93.4%-74.8%) in group V ($P = .185$; Table 3). The median time to engraftment was 17 days in group I; 18 days in groups II, III, and IV; and 19 days in group V. Engraftment was marginally delayed in group V compared with group I ($P = .053$). Additional HLA-DPB1 mismatching did not affect the cumulative incidence of engraftment in the 10 of 10 and 9 of 10 matched groups, respectively (Table 4). In multivariate analysis, blood type (ABO match or

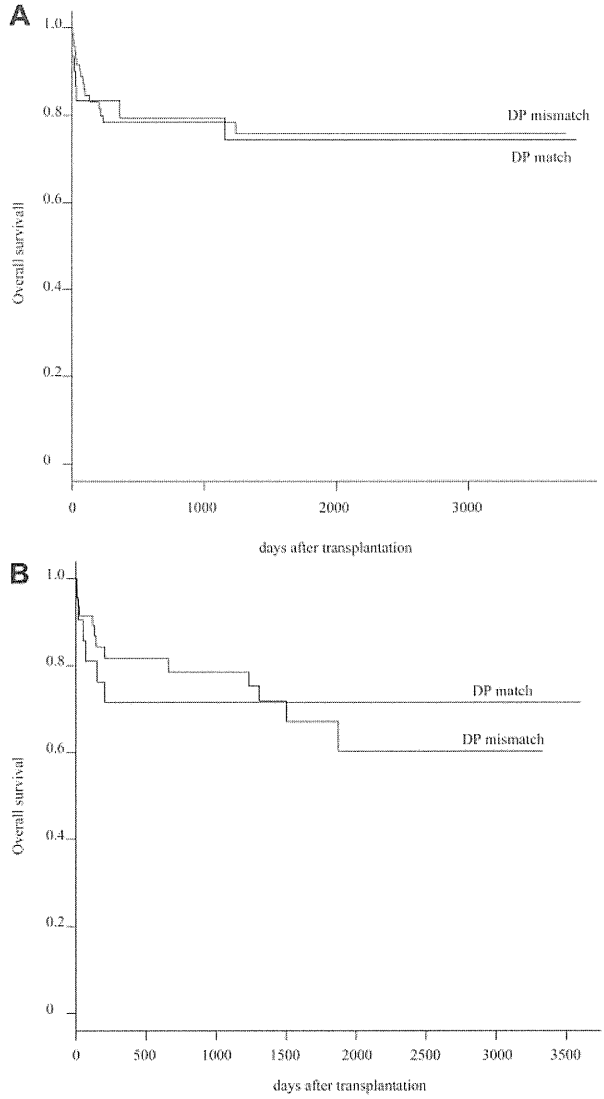

Figure 2. OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group. (A) Difference of OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group in 10 of 10 HLA allele matched pairs. (B) Difference of OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group in 9 of 10 HLA allele matched pairs.

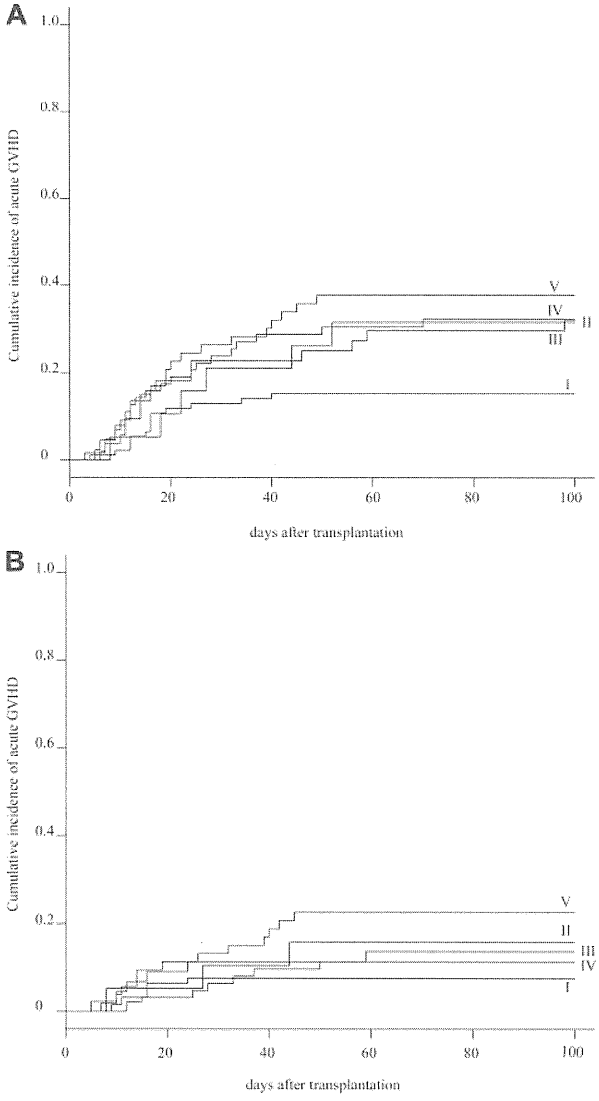

Figure 3. Cumulative incidence of acute GVHD. (A) Cumulative incidence of grade II-IV acute GVHD in 5 HLA groups. (B) Cumulative incidence of grade III-IV acute GVHD in 5 HLA groups.

minor mismatch: RR = 1.0; major mismatch or bidirection pair: RR = 5.102, P = .039) and HLA mismatching (group I: RR = 1.0; group V: RR = 4.906, P = .035) were significant risk factors for engraftment.

Acute GVHD

The cumulative incidence of acute GVHD at day 100 was evaluated in 272 patients. The cumulative incidence of grade II-IV and grade III-IV acute GVHD was 27.2% (95% CI, 32.5%-21.9%) and 12.9% (95% CI, 16.9%-8.9%) in the whole population, respectively (supplemental Figure 2). Subgroup analyses showed that the cumulative incidence of grades II-IV acute GVHD was statistically lower in group I (15.1%; 95% CI, 22.4%-7.8%) than in group V (37.7%; 95% CI, 50.9%-24.5%; P = .037), and marginally lower than in group III (31.8%; 95% CI, 45.8%-17.8%) and group IV (31.7%; 95% CI, 43.3%-20.1%; Table 3; Figure 3A). Whereas the cumulative incidence of grade III-IV acute GVHD was not significantly different among 5 groups: 7.5% (95% CI, 24.6%-0%) in group I, 15.8% (95% CI, 32.7%-0%) in group II, 13.6% (95% CI, 23.9%-3.3%) in group III, 11.1% (95% CI, 18.9%-3.3%) in group IV, and 22.6% (95% CI, 34.0%-11.2%) in group V (P = .139; Table 3; Figure 3B). Additional HLA-DPB1 mismatching evaluated in 155 patients did not affect the cumulative incidence of grade II-IV acute GVHD in the 10 of 10 and 9 of 10 matched groups, respectively (Table 4). Multivariate analysis showed that a significantly higher incidence of grade II-IV acute GVHD was associated with HLA mismatching (group I: RR = 1.0; group III: RR = 3.975, P = .002; group IV: RR = 3.334, P = .004; group V: RR = 3.665, P = .002). Other significant risk factors were the preconditioning regimen (Flu +CY + TBI/LFI ± ATG: RR = 1.0; TBI/LFI + CY: RR = 5.224, P = .003), and donor sex (female: RR = 1.0; male: RR = 1.844, P = .034; supplemental Table 3).

Chronic GVHD

The cumulative incidence of chronic GVHD at day 365 was evaluated in 232 patients. It was 24.5% (95% CI, 30.3%-18.7%) in the whole population. Subgroup analyses showed that it was comparable among the 5 HLA groups: 19.8% (95% CI, 28.8%-10.8%) in group I, 26.3% (95% CI, 49.3%-3.3%) in group II, 28.2% (95% CI, 43.3%-13.1%) in group III, 26.9% (95% CI, 39.2%-14.6%) in group IV, and 27.3% (95% CI, 42.1%-12.5%) in group V (P = .922; Table 3; supplemental Figure 3). HLA-DPB1 mismatching did not affect the cumulative incidence of chronic GVHD (Table 4).

Discussion

The survival rate in UBMT has increased substantially over the past 10 years in patients with SAA.[8-15] A 5-year survival rate of 90% has been reported in a small series of children.[16,17] A recent meta-analysis showed that detailed HLA-matching facilitated by DNA-based typing has contributed to the improved survival rate in patients with SAA who received an UBM transplant.[18] However, many patients with SAA who need hematopoietic stem cell transplantation do not have an HLA-complete matched donor. Our multivariate analysis indicated that among 4 HLA-mismatched groups, only HLA disparity of group V was a statistically significant unfavorable variable. We conclude that any type of HLA single-allele mismatch or multiple-allele mismatch within HLA-C and HLA class II (DRB1 or DQB1) is acceptable as an unrelated donor when an HLA complete match donor is unavailable.

We previously reported that HLA class I allele mismatching (HLA-A or -B) but not class II allele (HLA-DRB1) mismatching was a significant risk factor for survival when 6 alleles were analyzed.[4] HLA-A or -B mismatching pairs in the previous study were separated into 2 groups in the current study in which 10 alleles were analyzed. One group was a true single-allele mismatching pair of HLA-A or -B alleles (group II), and another was a multiple-allele mismatching pair of HLA-A or -B plus HLA-C and/or class II HLA alleles (group V). Because HLA-C and -DQB1 alleles were not typed, this type of multiple-allele mismatching might be mistaken as a single-allele mismatching pair, which was the reason for the inferior outcome of HLA-class I mismatching pairs in our previous study.

As the same in our previous study, mismatching of HLA-DRB1 did not provide a significant impact on clinical outcome. An HLA-DRB1 mismatching pair was also classified into a true single-allele mismatching of HLA-DRB1 (group III) and HLA-DRB1 plus HLA-C and/or HLA-DQB1 mismatching pairs (group IV). Interestingly, multiple mismatching of group IV was not

associated with increased mortality, which may explain why mismatching of HLA-DRB1 did not have a deleterious effect in the previous study.

The effect of HLA-DPB1 mismatching was also evaluated in HLA complete matched pairs (n = 101) and single-allele mismatched pairs (n = 69). The importance of DPB1 matching in the UBMT setting has been mainly discussed in patients with hematologic malignancies. Although results were controversial in early reports, recent studies support a significant effect of DPB1 mismatching on the incidence of acute GVHD, disease relapse, and OS.[19-22] In a large dataset of the International Histocompatibility Working Group, there was a statistically significant higher risk of both grade II-IV and grade III-IV acute GVHD.[19] The increased risk of acute GVHD was accompanied by a statistically significant decrease in disease relapse, probably because of the GVL effect, which offset the deleterious effect of acute GVHD. Survival rate was significantly better in DPB1-matched transplantations in patients with standard-risk leukemia but not in advanced leukemia. Conversely, in the HLA-mismatched group, there was a significant survival advantage in DPB1 mismatched pairs.

We expected that DPB1 matching might be beneficial for patients with AA who do not need the GVL effect. However, clinical outcomes, including incidence of acute GVHD, were not affected by DPB1 mismatching. HLA-DPB1 typing may not be essential to the donor selection algorithm for patients with SAA. Indeed, HLA-DPB1 mismatching was observed in 74% of recipient/donor pairs, and it may be practically difficult to find HLA 12 of 12 matched donors.

In conclusion, this retrospective study confirms the importance of HLA matching between recipients and donors to improve the outcome of UBMT for patients with SAA patients. However, this study showed that only 33% of patients received transplants from an HLA 10 of 10 matched donor. The availability of unrelated hematopoietic stem cell transplants can be increased through the judicious selection of donors with HLA mismatches that do not substantially lower survival.

Authorship

Contribution: H. Yagasaki analyzed the data and wrote the paper; S. Kojima designed the research and analyzed the data; and H. Yabe, K.K., H.K., H.S., M.T., S. Kato, T.K., Y.M., and Y.K performed and supervised the research.

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References

1. Hows J, Szydlo R, Anasetti C, Camitta B, Gajewski J, Gluckman E. Unrelated donor marrow transplants for severe acquired aplastic anemia. *Bone Marrow Transplant.* 1992;10(suppl 1): 102-106.
2. Deeg HJ, Seidel K, Casper J, et al. Marrow transplantation from unrelated donors for patients with severe aplastic anemia who have failed immunosuppressive therapy. *Biol Blood Marrow Transplant.* 1999;5(4):243-252.
3. Passweg JR, Pérez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transplant.* 2006;37(7):641-649.
4. Kojima S, Matsuyama T, Kato S, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood.* 2002; 100(3):799-803.
5. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood.* 2004;104(7):1923-1930.
6. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood.* 2002;99(11):4200-4206.
7. Morishima Y, Yabe T, Matsuo K, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. *Biol Blood Marrow Transplant.* 2007;13(3):315-328.
8. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood.* 2007;110(13): 4576-4583.
9. Kim SY, Lee JW, Lim J, et al. Unrelated donor bone marrow transplants for severe aplastic anemia with conditioning using total body irradiation and cyclophosphamide. *Biol Blood Marrow Transplant.* 2007;13(7):863-867.
10. Perez-Albuerne ED, Eapen M, Klein J, et al. Outcome of unrelated donor stem cell transplantation for children with severe aplastic anemia. *Br J Haematol.* 2008;141(2):216-223.
11. Maury S, Balere-Appert ML, Chir Z, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. *Haematologica.* 2007;92(5): 589-96.
12. Viollier R, Socie G, Tichelli A, et al. Recent improvement in outcome of unrelated donor transplantation for aplastic anemia. *Bone Marrow Transplant.* 2008;41(1):45-50.
13. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant.* 2005;36(11):947-950.
14. Deeg HJ, O'Donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood.* 2006;108(5):1485-1491.
15. Lee JW, Cho BS, Lee SE, et al. The outcome of unrelated hematopoietic stem cell transplants with total body irradiation (800 cGy) and cyclophosphamide (120 mg/kg) in adult patients with acquired severe aplastic anemia. *Biol Blood Marrow Transplant.* 2011;17(1):101-108.
16. Yagasaki H, Takahashi Y, Hama A, et al. Comparison of matched-sibling donor BMT and unrelated donor BMT in children and adolescent with acquired severe aplastic anemia. *Bone Marrow Transplant.* 2010;45(10):1508-1513.
17. Kennedy-Nasser AA, Leung KS, Mahajan A, et al. Comparable outcomes of matched-related and alternative donor stem cell transplantation for pediatric severe aplastic anemia. *Biol Blood Marrow Transplant.* 2006;12(12):1277-1284.
18. Peinemann F, Grouven U, Kröger N, Pittler M, Zschorlich B, Lange S. Unrelated donor stem cell transplantation in acquired severe aplastic anemia: a systematic review. *Haematologica.* 2009; 94(12):1732-1742.
19. Shaw BE, Gooley TA, Malkki M, et al. The importance of HLA-DPB1 in unrelated donor hematopoietic cell transplantation. *Blood.* 2007;110(13): 4560-4566.
20. Kawase T, Matsuo K, Kashiwase K, et al. HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism. *Blood.* 2009;113(12):2851-2285.
21. Crocchiolo R, Zino E, Vago L, et al. Nonpermissive HLA-DPB1 disparity is a significant independent risk factor for mortality after unrelated hematopoietic stem cell transplantation. *Blood.* 2009; 114(7):1437-1444.
22. Shaw BE, Mayor NP, Russell NH, et al. Diverging effects of HLA-DPB1 matching status on outcome following unrelated donor transplantation depending on disease stage and the degree of matching for other HLA alleles. *Leukemia.* 2010;24(1):58-65.

GUIDELINES

Recommended Screening and Preventive Practices for Long-Term Survivors after Hematopoietic Cell Transplantation

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Advances in hematopoietic cell transplantation (HCT) technology and supportive care techniques have led to improvements in long-term survival after HCT. Emerging indications for transplantation, introduction of newer graft sources (eg, umbilical cord blood) and transplantation of older patients using less intense conditioning regimens have also contributed to an increase in the number of HCT survivors. These survivors are at risk for developing late complications secondary to pre-, peri-, and posttransplantation exposures and risk factors. Guidelines for screening and preventive practices for HCT survivors were published in 2006. An international group of transplantation experts was convened in 2011 to review contemporary literature and update the recommendations while considering the changing practice of transplantation and international applicability of these guidelines. This review provides the updated recommendations for screening and preventive practices for pediatric and adult survivors of autologous and allogeneic HCT.

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INTRODUCTION

Approximately 50,000 people undergo hematopoietic cell transplantation (HCT) worldwide each year. Advances in transplantation techniques and supportive care practices have led to progressive improvements in survival for HCT recipients. As patients survive long term after transplantation, they are at risk for developing late complications related to pre-, peri-, and posttransplantation exposures. These complications can cause substantial morbidity, impair quality of life, and can contribute to late mortality in HCT recipients. Several studies have shown that the life

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expectancy of HCT survivors is lower than expected at 10 to 30 years posttransplantation and secondary cancers, infections, and organ dysfunction are common causes of late deaths in this population [1-6].

Recognizing the need for guidance about appropriate systematic long-term follow-up of HCT survivors, the Center for International Blood and Marrow Transplant Research (CIBMTR), the European Group for Blood and Marrow Transplantation, and the American Society for Blood and Marrow Transplantation convened a group of experts in 2006 and provided consensus recommendations for screening and preventive practices for autologous and allogeneic HCT survivors [7,8]. To update these previous guidelines, the international working group was reconvened in 2011 to review the prevailing literature in late effects of transplantation and to suggest revised guidelines, if applicable. To ensure international applicability, the working group included participants from the Asia-Pacific Blood and Marrow Transplantation Group, the Bone Marrow Transplant Society of Australia and New Zealand, the East Mediterranean Blood and Marrow Transplantation Group, and Sociedade Brasileira de Transplante de Medula Ossea.

The proposed guidelines focus on risks faced by children and adults who have survived 6 months or more following transplantation and address autologous and allogeneic HCT recipients. Since long-term HCT recipients may no longer be under the care of transplant centers and may have returned to the care of community health care providers, the guidelines are geared towards providers who routinely care for HCT recipients as well as those who do not.

The working group recognized the general lack of clinical trials focused on screening and preventive practices among HCT recipients and the need for more research in this area. Hence, many of these recommendations are not based on evidence derived from randomized or other controlled trials but are supported by retrospective studies that have identified specific complications in long-term survivors and their associated risk-factors. When such studies are not available, the guidelines are based on knowledge derived from non-transplant patients as well as on the consensus opinion of the working group participants. Taking into account the risks and potential consequences of late complications, they represent sensible practices to optimize outcomes. The recommendations should not be interpreted as mandatory for all recipients; good medical practice and judgment dictate that certain recommendations may not be applicable or may even be contraindicated in individual patients or groups of patients.

It was also recognized that the practice of HCT is continuously changing. Some examples of such changes include emerging indications for transplantation (e.g. autoimmune diseases, sickle cell disease), increased utilization of newer donor sources (e.g. umbilical cord blood and haploidentical donors), decreased use of total body irradiation (TBI) for conditioning and evaluation of novel therapies as part of HCT (e.g. post-transplant maintenance therapy in myeloma). With the advent of non-myeloablative and reduced intensity conditioning (NMA/RIC) regimens, a larger number of older patients now receive transplantation. The risks and constellation of late complications may change as newer practices in transplantation become more prevalent. Providers should be cognizant of any unique exposures and risks associated with these practices (e.g. delayed immune reconstitution in umbilical cord blood recipients) when considering a long-term followup care plan for their patients.

A broad constellation of medical issues faced by late survivors of transplantation is presented. Most of the late complications discussed here pertain particularly to allogeneic recipients. However, autologous recipients are at risk for many of the same late complications and may experience unusual toxicity or immune impairment following transplantation that places them at risk similar to allogeneic recipients (e.g. exposure to prolonged corticosteroids or other drugs that may cause prolonged lymphopenia post-transplantation). Therefore, although some of the following recommendations do not generally apply to autologous recipients, providers should remain alert to these complications in all patients.

The guidelines are summarized in Tables 1 and 2. The Supplementary Tables includes tables that highlight recommendations for post-transplant immunizations (Appendix Table A) and recommendations by selected exposures/risk-factors (TBI, chronic GVHD, pediatric recipients) (Appendix Table B). Appendix Table C lists other guidelines that have been referenced in this manuscript along with current links to their website. Readers can also refer to guidelines developed by the Children's Oncology Group for followup for pediatric cancer survivors, which include information on pediatric HCT recipients (www.survivorshipguidelines.org). Representative references are included in this document to guide readers who would like more information on individual topics.

The National Marrow Donor Program (NMDP) publishes a patient version of the followup guidelines (www.BeTheMatch.org/Patient); we recommend that patients use these guidelines to establish a long-term followup care plan in consultation with their health care provider based on their individual exposures and risk factors. The NMDP also makes a summary of the guidelines available for physicians, (online, mobile app, and in print at www.marrow.org/md-guidelines).

Table 1. Summary Recommendations for Screening and Prevention of Late Complications in Long-Term HCT Survivors

Tissues/Organs	Late Complications	General Risk Factors	Monitoring Tests	Monitoring Tests and Preventive Measures on All HCT Recipients	Monitoring Tests and Preventive Measures in Special Populations
Immune system	-Infections	-Donor source -HLA disparity -T cell depletion -GVHD -Prolonged immunosuppression -Venous access devices	-CMV antigen or PCR in patients at high risk for CMV reactivation	-PCP prophylaxis for initial 6 months after HCT -Immunizations posttransplantation according to published guidelines -Administration of antibiotics for endocarditis prophylaxis according to American Heart Association guidelines	-Patients with cGVHD: Antimicrobial prophylaxis targeting encapsulated organisms and PCP for the duration of immunosuppressive therapy -Patients with cGVHD: Screening for CMV reactivation should be based on risk factors, including intensity of immunosuppression.
Ocular	-Cataracts -Sicca syndrome -Microvascular retinopathy	-TBI/radiation exposure to head and neck -Corticosteroids -GVHD	-Ophthalmologic exam	-Routine clinical evaluation at 6 months and 1 year after HCT and at least yearly thereafter -Ophthalmologic examination with measurement of visual acuity and fundus examination at 1 year after HCT, subsequent evaluation based on findings and risk factors -Prompt ophthalmologic examination in patients with visual symptoms	-Patients with cGVHD: Routine clinical evaluation, and if indicated, ophthalmologic examination more frequently
Oral	-Sicca syndrome -Caries	-GVHD -TBI/radiation exposure to head and neck	-Dental assessment	-Education about preventive oral health practices -Clinical oral assessment at 6 months and 1 year after HCT and at least yearly thereafter with particular attention to intraoral malignancy evaluation -Dental assessment at 1 year after HCT and then at least yearly thereafter	-Pediatric recipients: Yearly assessment of teeth development -Patients with cGVHD: Consider more frequent oral and dental assesments with particular attention to intraoral malignancy evaluation
Respiratory	-Idiopathic pneumonia syndrome -Bronchiolitis obliterans syndrome -Cryptogenic organizing pneumonia -Sinopulmonary infections	-TBI/radiation exposure to chest -GVHD -Infectious agents -Allogeneic HCT -Busulfan exposure	-PFTs -Radiologic studies (eg, chest x-ray, CT scan)	-Routine clinical evaluation at 6 months and 1 year after HCT and at least yearly thereafter -Assessment of tobacco use and couselling against smoking -PFTs and focused radiologic assessment for allogeneic HCT recipients with symptoms or signs of lung compromise	-Patients with cGVHD: Some experts recommend earlier and more frequent clinical evaluation and PFTs
Cardiac and vascular	-Cardiomyopathy -Congestive heart failure -Arrhythmias -Valvular anomaly -Coronary artery disease -Cerebrovascular disease -Peripheral arterial disease	-Anthracycline exposure -TBI/radiation exposure to neck or chest -Older age at HCT -Allogeneic HCT -Cardiovascular risk factors before/after HCT -Chronic kidney disease -Metabolic syndrome	-Cumulative dose of anthracyclines -Echocardiogram with ventricular function, ECG in patients at risk and in symptomatic patients -Fasting lipid profile (including HDL-C, LDL-C and triglycerides) -Fasting blood sugar	-Routine clinical assessment of cardiovascular risk factors as per general health maintenance at 1 year and at least yearly thereafter -Education and counseling on "heart-healthy" lifestyle (regular exercise, healthy weight, no smoking, dietary counseling) -Early treatment of cardiovascular risk factors such as diabetes, hypertension, and dyslipidemia -Administration of antibiotics for endocarditis prophylaxis according to American Heart Association guidelines	

Liver	-GVHD -Hepatitis B -Hepatitis C -Iron overload	-Cumulative transfusion exposure -Risk factors for viral hepatitis transmission	-LFTs -Liver biopsy -Serum ferritin -Imaging for iron overload (MRI or SQUID)	-LFTs every 3-6 months in the first year, then individualized, but at least yearly thereafter -Monitor viral load by PCR for patients with known hepatitis B or C, with liver and infectious disease specialist consultation -Consider liver biopsy at 8-10 years after HCT to assess cirrhosis in patients with chronic HCV infection -Serum ferritin at 1 year after HCT in patients who have received RBC transfusions; consider liver biopsy or imaging study for abnormal results based on magnitude of elevation and clinical context; subsequent monitoring is suggested for patients with elevated LFTs, continued RBC transfusions, or presence of HCV infection	
Renal and genitourinary	-Chronic kidney disease -Bladder dysfunction -Urinary tract infections	-TBI -Drug exposure (eg, calcineurin inhibitors, amphotericin, aminoglycosides) -CMV -Hemorrhagic cystitis	-Urine protein -Serum creatinine -BUN	-Blood pressure assessment at every clinic visit, with aggressive hypertension management -Assess renal function with BUN, creatinine and urine protein at 6 months, 1 year and at least yearly thereafter -Consider further workup (kidney biopsy or renal ultrasound) for for further workup of renal dysfunction as clinically indicated	
Muscle and connective tissue	-Myopathy -Fascitis/scleroderma -Polymyositis	-Corticosteroids -GVHD	-Evaluate ability to stand from a sitting position -Clinical evaluation of joint range of motion	-Follow general population guidelines for physical activity -Frequent clinical evaluation for myopathy in patients on corticosteroids	-Patients with cGVHD: Physical therapy consultation in patients with prolonged corticosteroid exposure, fascitis, or scleroderma -Patients with cGVHD: Frequent clinical evaluation by manual muscle tests or by assessing ability to go from sitting to standing position for patients on prolonged corticosteroids
Skeletal	-Osteopenia/ osteoporosis -Avascular necrosis	-Inactivity -TBI -Corticosteroids -GVHD -Hypogonadism -Allogeneic HCT	-Dual photon densitometry -MRI to evaluate patients with joint symptoms	-Dual photon densitometry at 1 year for adult women, all allogeneic HCT recipients and patients who are at high risk for bone loss; subsequent testing determined by defects or to assess response to therapy -Physical activity, vitamin D, and calcium supplementation to prevent loss of bone density	-Patients with cGVHD: Consider dual photon densitometry at an earlier date in patients with prolonged corticosteroid or calcineurin inhibitor exposure.
Nervous system	-Leukoencephalopathy -Late infections -Neuropsychological and cognitive deficits -Calcineurin neurotoxicity -Peripheral neuropathy	-TBI/radiation exposure to head -GVHD -Exposure to fludarabine -Intrathecal chemotherapy	-	-Clinical evaluation for symptoms and signs of neurologic dysfunction at 1 year and yearly thereafter -Diagnostic testing (eg, radiographs, nerve conduction studies) for those with symptoms or signs	-Pediatric recipients: Annual assessment for congnitive development milestones

(Continued)