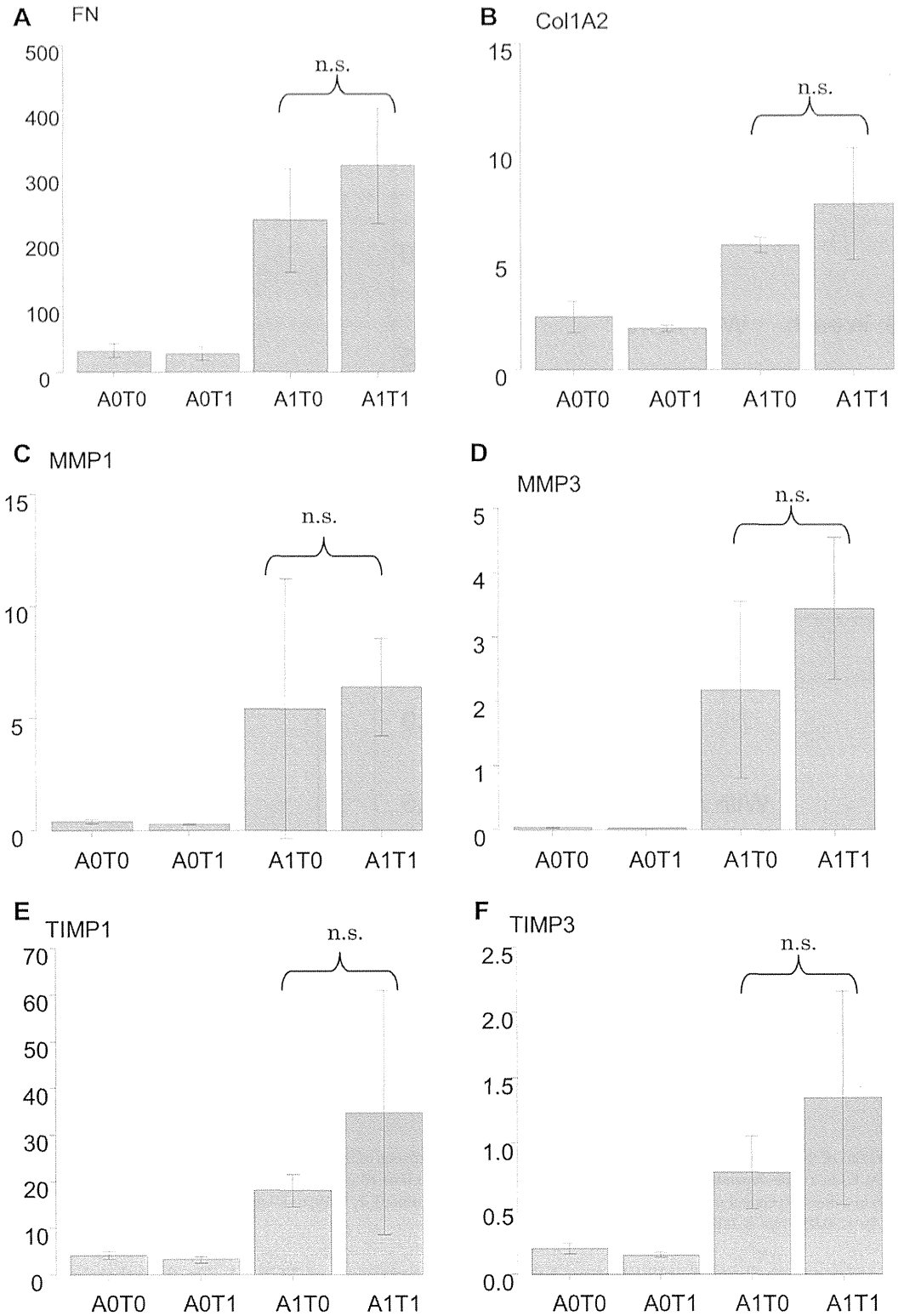

Figure 6. Comparisons of the relative transcripts of extracellular matrix and metalloproteinase families with or without blocking of the TGF-βR2 pathway. The expression of **(A)** fibronectin 1 (FN1), **(B)** collagen type1 α 2 (COL1A2), **(C)** MMP-1, **(D)** MMP-3, **(E)** TIMP-1, and **(F)** TIMP-3 were evaluated with qRT-PCR and compared in cells without both adiponectin and anti–TGF-βR2 antibody (A0T0), without adiponectin but with 20 μg/mL of anti–TGF-βR2 antibody (A0T1), with 20 μg/mL of adiponectin but without anti–TGF-βR2 antibody (A1T0), and with 20 μg/mL of both adiponectin and anti–TGF-βR2 antibody (A1T1) at 3 days after administration.

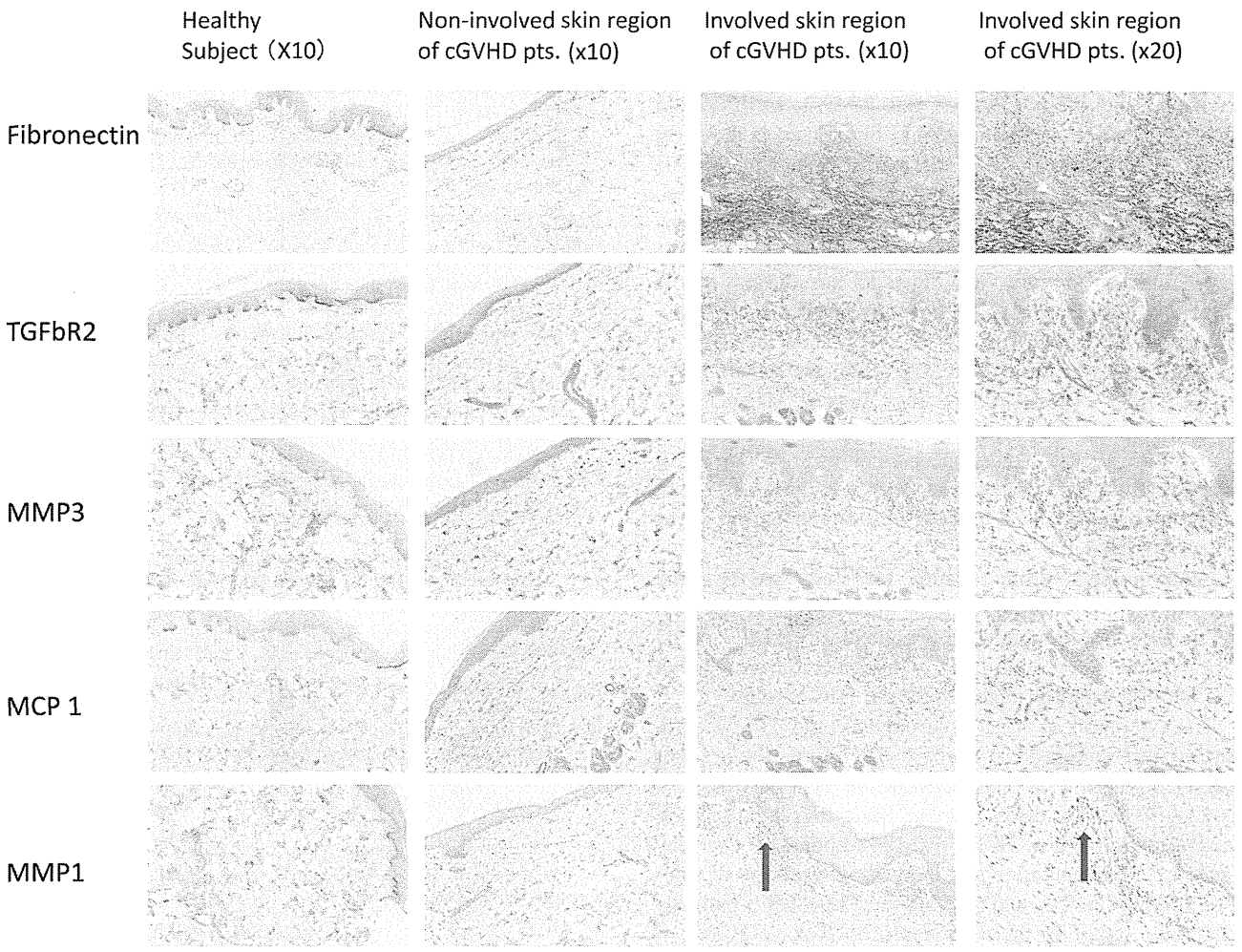

Figure 7. Immunohistochemistry was performed using formalin-fixed paraffin-embedded skin samples of a healthy subject and a patient with skin cGVHD for fibronectin, TGF-βR2, MMP-3, MCP-1, and MMP-1. EnVision immunohistochemistry stain. The blue arrows indicate positive regions for MMP-1.

Table 1. Summary of other investigations that have assessed the effects of adiponectin on both MMPs and TIMPs[a]

Adiponectin isoform	Target cell	Matrix metalloproteinase (MMP)	Tissue inhibitor of metalloproteinase (TIMP)	Reference
Full-length adiponectin, trimers	—	MMP-9 gene expression ↑	TIMP-1 expression ↑	[35]
In vivo study using knockout vs. wild type mice	Rat and mouse cardiomyocyte	ROS-induced MMP-2 and MMP-9 activity ↓	MMP-2-to-TIMP-2 and MMP-9-to-TIMP-1 ratios ↑ in knockout mice	[36]
Full-length adiponectin, trimers	Human chondrocytes of osteoarthritis	MMP-1, MMP-3, and MMP-13 expression and secretion ↑	TIMP-1 expression, no change	[37]
No details	Human trophoblast	MMP-2 and MMP-9 activity ↑	TIMP-1 expression, no change TIMP-2 expression ↓	[38]
No details	Rat hepatic stellate cells	MMP-1 activity ↑	Leptin-stimulated TIMP-1 ↓	[39]
Full-length adiponectin, Trimers	Human and murine chondrocytes	MMP-3 and MMP-9 secretion ↑ MMP-2 secretion, no change	TIMP-1 secretion, no change	[40]
Full-length adiponectin, trimers	Human chondrocyte	IL-β–induced MMP-13 expression ↑ MMP-3 expression, no change	TIMP-2 expression ↑ TIMP-1 expression, no change	[41]
No details	Human monocyte -derived macrophages	MMP-9 expression, no change	TIMP-1 expression ↑	[42]

[a]Data from http://www.ncbi.nlm.nih.gov/pubmed/, using the search terms *adiponectin*, *MMP*, and *TIMP*.

HMW-/MMW-adiponectin not only induced the synthesis and deposition of ECMs; it also upregulated the expression of both TIMPs and MMPs. These findings are consistent with the observation that TIMP-1, MMP-1, and MMP-3 are all increased in dermal fibroblasts in the early stages of systemic sclerosis, whereas MMP-1 and MMP-3 are decreased in the late stages [26]. Taken together, these findings suggest that HMW-/MMW-adiponectin can modulate dermal fibrotic pathways. However, the current findings were obtained in vitro, and thus do not directly show fibrosis in vivo by HMW-/MMW-adiponectin. In fact, the IHC of skin cGVHD actually showed certain increases in the expressions of fibronectin, TGF-βR2, and MMP-3, but not of TIMPs. These IHC findings suggest that cGVHD could not be explained only by adiponectin, although skin biopsy samples from only one patient were too small to establish a definite conclusion. The association between adiponectin and skin cGVHD scores should be evaluated in future prospective trials.

Other possible limitations of our study are that the assessment time of the current fibroblast analysis was different from the actual development of skin cGVHD and that not only long-term steroid administration but also autopsy samples might affect our IHC results.

The symptoms of cGVHD are diverse and complicated, beyond just simple skin fibrosis. Therefore, the role of HMW-adiponectin in the network of cGVHD in vivo remains to be elucidated. Further basic investigations are needed to clarify how HMW-/MMW-adiponectin can play a role in ECM regulation and the pathophysiology of sclerotic cGVHD in vivo, and whether the adiponectin-pathway could be a target for the treatment of sclerotic cGVHD.

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Conflict of interest disclosure

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

References

1. Paczesny S, Hanauer D, Sun Y, Reddy P. New perspectives on the biology of acute GVHD. Bone Marrow Transplant. 2010;45:1–11.
2. Sarantopoulos S, Stevenson KE, Kim HT, et al. Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. Blood. 2009;113:3865–3874.
3. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, von Bergwelt-Baildon MS. The role of B cells in the pathogenesis of graft-versus-host disease. Blood. 2009;114:4919–4927.
4. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009;373:1550–1561.
5. Pidala J, Anasetti C, Jim H. Quality of life after allogeneic hematopoietic cell transplantation. Blood. 2009;114:7–19.
6. Schultz KR, Miklos DB, Fowler D, et al. Toward biomarkers for chronic graft-versus-host disease: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: III. Biomarker Working Group Report. Biol Blood Marrow Transplant. 2006;12:126–137.
7. Fujii H, Cuvelier G, She K, et al. Biomarkers in newly diagnosed pediatric-extensive chronic graft-versus-host disease: a report from the Children's Oncology Group. Blood. 2008;111:3276–3285.
8. Imamura M, Hashino S, Kobayashi H, et al. Serum cytokine levels in bone marrow transplantation: synergistic interaction of interleukin-6, interferon-gamma, and tumor necrosis factor-alpha in graft-versus-host disease. Bone Marrow Transplant. 1994;13:745–751.
9. Liem LM, van Houwelingen HC, Goulmy E. Serum cytokine levels after HLA-identical bone marrow transplantation. Transplantation. 1998;66:863–871.
10. Yamauchi T, Kadowaki T. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. Int J Obes (Lond). 2008; 32(Suppl 7):S13–S18.
11. Stofkova A. Leptin and adiponectin: from energy and metabolic dysbalance to inflammation and autoimmunity. Endocr Regul. 2009;43:157–168.
12. Fantuzzi G. Adiponectin and inflammation: consensus and controversy. J Allergy Clin Immunol. 2008;121:326–330.
13. Tilg H, Moschen AR. Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. Clin Sci (Lond). 2008;114:275–288.
14. Otero M, Lago R, Gomez R, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis. 2006;65:1198–1201.
15. Yamamoto K, Kiyohara T, Murayama Y, et al. Production of adiponectin, an anti-inflammatory protein, in mesenteric adipose tissue in Crohn's disease. Gut. 2005;54:789–796.
16. Brochu-Gaudreau K, Rehfeldt C, Blouin R, Bordignon V, Murphy BD, Palin MF. Adiponectin action from head to toe. Endocrine. 2010;37: 11–32.
17. Neumann E, Frommer KW, Vasile M, Muller-Ladner U. Adipocytokines as driving forces in rheumatoid arthritis and related inflammatory diseases? Arthritis Rheum. 2011;63:1159–1169.
18. Nakasone H, Binh PN, Yamazaki R, et al. Association between serum high-molecular-weight adiponectin level and the severity of chronic graft-versus-host disease in allogeneic stem cell transplantation recipients. Blood. 2011;117:3469–3472.
19. Jinnin M. Mechanisms of skin fibrosis in systemic sclerosis. J Dermatol. 2010;37:11–25.
20. Cotton SA, Herrick AL, Jayson MI, Freemont AJ. TGF beta–a role in systemic sclerosis? J Pathol. 1998;184:4–6.
21. Tsang ML, Zhou L, Zheng BL, et al. Characterization of recombinant soluble human transforming growth factor-beta receptor type II (rhTGF-beta sRII). Cytokine. 1995;7:389–397.
22. Lareu RR, Subramhanya KH, Peng Y, et al. Collagen matrix deposition is dramatically enhanced in vitro when crowded with charged macromolecules: the biological relevance of the excluded volume effect. FEBS Lett. 2007;581:2709–2714.
23. Chen CZ, Peng YX, Wang ZB, et al. The Scar-in-a-Jar: studying potential antifibrotic compounds from the epigenetic to extracellular level in a single well. Br J Pharmacol. 2009;158:1196–1209.

24. Karamichos D, Guo XQ, Hutcheon AE, Zieske JD. Human corneal fibrosis: an in vitro model. Invest Ophthalmol Vis Sci. 2010;51: 1382–1388.
25. Ivanov D, Philippova M, Antropova J, et al. Expression of cell adhesion molecule T-cadherin in the human vasculature. Histochem Cell Biol. 2001;115:231–242.
26. Kuroda K, Shinkai H. Gene expression of types I and III collagen, decorin, matrix metalloproteinases and tissue inhibitors of metalloproteinases in skin fibroblasts from patients with systemic sclerosis. Arch Dermatol Res. 1997;289:567–572.
27. Salmela MT, Karjalainen-Lindsberg ML, Jeskanen L, Saarialho-Kere U. Overexpression of tissue inhibitor of metalloproteinases-3 in intestinal and cutaneous lesions of graft-versus-host disease. Mod Pathol. 2003;16:108–114.
28. Ebina K, Fukuhara A, Ando W, et al. Serum adiponectin concentrations correlate with severity of rheumatoid arthritis evaluated by extent of joint destruction. Clin Rheumatol. 2009;28:445–451.
29. Hadjadj S, Aubert R, Fumeron F, et al. Increased plasma adiponectin concentrations are associated with microangiopathy in type 1 diabetic subjects. Diabetologia. 2005;48:1088–1092.
30. Rovin BH, Song H, Hebert LA, et al. Plasma, urine, and renal expression of adiponectin in human systemic lupus erythematosus. Kidney Int. 2005;68:1825–1833.
31. Fayad R, Pini M, Sennello JA, et al. Adiponectin deficiency protects mice from chemically induced colonic inflammation. Gastroenterology. 2007;132:601–614.
32. Ehling A, Schaffler A, Herfarth H, et al. The potential of adiponectin in driving arthritis. J Immunol. 2006;176:4468–4478.
33. Masui Y, Asano Y, Shibata S, et al. Serum adiponectin levels inversely correlate with the activity of progressive skin sclerosis in patients with diffuse cutaneous systemic sclerosis. J Eur Acad Dermatol Venereol. 2011;26:354–360.
34. Smalls LK, Randall Wickett R, Visscher MO. Effect of dermal thickness, tissue composition, and body site on skin biomechanical properties. Skin Res Technol. 2006;12:43–49.
35. Wanninger J, Walter R, Bauer S, et al. MMP-9 activity is increased by adiponectin in primary human hepatocytes but even negatively correlates with serum adiponectin in a rodent model of non-alcoholic steatohepatitis. Exp Mol Pathol. 2011;91:603–607.
36. Essick EE, Ouchi N, Wilson RM, et al. Adiponectin mediates cardioprotection in oxidative stress-induced cardiac myocyte remodeling. Am J Physiol Heart Circ Physiol. 2011;301:H984–H993.
37. Kang EH, Lee YJ, Kim TK, et al. Adiponectin is a potential catabolic mediator in osteoarthritis cartilage. Arthritis Res Ther. 2010; 12:R231.
38. Benaitreau D, Dos Santos E, Leneveu MC, et al. Effects of adiponectin on human trophoblast invasion. J Endocrinol. 2010;207:45–53.
39. Handy JA, Saxena NK, Fu P, et al. Adiponectin activation of AMPK disrupts leptin-mediated hepatic fibrosis via suppressors of cytokine signaling (SOCS-3). J Cell Biochem. 2010;110:1195–1207.
40. Lago R, Gomez R, Otero M, et al. A new player in cartilage homeostasis: adiponectin induces nitric oxide synthase type II and pro-inflammatory cytokines in chondrocytes. Osteoarthritis Cartilage. 2008;16:1101–1109.
41. Chen TH, Chen L, Hsieh MS, Chang CP, Chou DT, Tsai SH. Evidence for a protective role for adiponectin in osteoarthritis. Biochim Biophys Acta. 2006;1762:711–718.
42. Kumada M, Kihara S, Ouchi N, et al. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation. 2004;109:2046–2049.
43. Ezure T, Amano S. Adiponectin and leptin up-regulate extracellular matrix production by dermal fibroblasts. Biofactors. 2007;31:229–236.
44. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48: 452–458.

Changes in the Clinical Impact of High-Risk Human Leukocyte Antigen Allele Mismatch Combinations on the Outcome of Unrelated Bone Marrow Transplantation

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A B S T R A C T

Several high-risk HLA allele mismatch combinations (HR-MMs) for severe acute graft-versus-host disease (GVHD) have been identified by analyzing transplantation outcomes in Japanese unrelated hematopoietic stem cell transplant recipients. In this study, we analyzed the effects of HR-MMs in 3 transplantation time periods. We confirmed that the incidence of grade III to IV acute GVHD in the HR-MM group was significantly higher than that in the low-risk (LR) MM group (hazard ratio [HR], 2.74; $P < .0001$) in the early time period (1993 to 2001). However, the difference in the incidence of grade III to IV acute GVHD between the HR-MM and LR-MM groups was not statistically significant (HR, 1.06; $P = .85$ and HR, .40; $P = .21$, respectively) in the mid (2002 to 2007) and late (2008 to 2011) time periods. Similarly, survival in the HR-MM group was significantly inferior to that in the LR-MM group (HR, 1.46; $P = .019$) in the early time period, whereas the difference in survival between the 2 groups was not statistically significant in the mid and late time periods (HR, 1.06; $P = .75$ and HR, .82; $P = .58$, respectively). In conclusion, the adverse impact of HR-MM has become less significant over time. Unrelated transplantation with a single HR-MM could be a viable option in the absence of a matched unrelated donor or an unrelated donor with a single LR-MM.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from an unrelated donor has been established as an effective treatment option for patients with hematological diseases who lack a human leukocyte antigen (HLA)–matched related donor. However, an HLA mismatch at the genetic level (allele mismatch) may be observed even in HSCT from a serologically HLA-matched donor (antigen match), and the presence of an allele mismatch adversely affects the incidence of severe acute graft-versus-host disease (GVHD) and survival [1-4]. We recently showed that the presence of single HLA allele mismatches at the HLA-A, -B, -C, or -DRB1 loci equivalently affect the outcome of HSCT, although a previous study from Japan reported that an HLA-A or -B allele mismatch impairs overall survival more strongly than an HLA-C or -DRB1 allele mismatch [4,5]. These findings suggest that the

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Table 1
Patient Characteristics

Characteristic	Match n = 2504			Low-Risk Mismatch n = 1057			High-Risk Mismatch n = 157		
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
	802	814	888	412	351	294	64	71	22
Age (recipient)									
Median	32	38	43	31	38	43	33	39	41
Age (donor)									
Median	34	34	36	33	34	37	35	36	37
Sex (recipient)									
Female	292	305	378	162	165	123	27	27	9
Male	510	509	510	250	186	171	37	44	13
Sex (donor)									
Female	286	262	266	164	158	107	20	28	5
Male	512	548	622	247	190	187	43	43	17
N.A.	4	4	0	1	3	0	1	0	0
Sex mismatch									
Match	507	537	512	238	209	166	35	40	14
Male to female	148	158	244	85	72	72	17	15	6
Female to male	143	115	132	88	67	56	11	16	2
N.A.	4	4	0	1	3	0	1	0	0
ABO blood type									
Match	454	462	500	167	151	121	33	31	9
Minor mismatch	154	162	175	112	84	81	15	18	3
Major mismatch	125	114	142	82	67	61	9	18	4
Bidirectional mismatch	58	70	71	45	46	31	7	4	6
N.A.	11	6	0	6	3	0	0	0	0
Disease									
AML	269	415	495	134	168	170	15	29	12
ALL	229	229	249	116	96	76	11	23	8
CML	237	84	29	125	42	14	30	3	0
MDS	67	86	115	37	45	34	8	16	2
Disease risk									
Low	552	533	607	265	219	181	40	38	12
High	230	239	280	135	116	113	21	28	10
Others	20	42	1	12	16	0	3	5	0
Cell dose (cells/kg)									
Median	3.0	2.7	2.7	3.0	2.6	2.6	3.1	2.8	2.6
GVHD prophylaxis									
CSA-based	545	306	185	267	114	47	45	21	2
TAC-based	240	499	689	135	227	240	19	50	20
N.A.	17	9	14	10	10	7	0	0	0
Conditioning regimen									
TBI regimen	760	639	560	394	272	194	59	53	15
Non-TBI regimen	30	114	328	17	52	100	3	11	7
N.A.	12	61	0	1	27	0	2	7	0

N.A. indicates not available; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus; TBI, total body irradiation.

clinical impact of an HLA mismatch may have changed over time periods.

Some investigators have tried to identify specific donor-recipient allele combinations that may be associated with a higher risk of severe acute GVHD [6,7]. Kawase et al. found 16 high-risk HLA allele mismatch combinations (HR-MMs) for severe acute GVHD [7]. They also showed that the number of HR-MMs was associated with severe GVHD and poor survival, whereas the presence of mismatch combinations other than HR-MMs (low-risk mismatch combinations, LR-MMs) did not affect the outcome of HSCT. However, their study included a variety of benign and malignant hematological diseases. In addition, they included donor-recipient pairs with more than 1 HLA mismatch. The impact of each specific mismatch combination was evaluated after adjusting for the number of HLA mismatches in other loci in a multivariate model, but the possible presence of HR-MMs in other loci or the interaction between HLA mismatch combinations could not be appropriately treated in their model. At that time, the study design was inevitable, because the number of each HLA mismatch combination was limited. However, several years have passed and the amount of unrelated HSCT data in the Transplant Registry Unified Management Program (TRUMP) has increased to more than 13,500 donor-recipient pairs. Therefore, in this study, we reanalyzed the impact of HR-MMs, excluding HSCT with multiple HLA mismatches in patients with relatively homogeneous background diseases. In addition, we evaluated the impact of HLA mismatch on transplantation outcomes considering the period effect, because the impact of HR-MM mismatch might have changed over time periods, as we previously reported in an analysis of single HLA allele mismatches at the HLA-A, -B, -C, and -DRB1 loci [5].

METHODS

Patients

Patients aged at least 16 years with acute myeloblastic leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, or chronic myelogenous leukemia (CML) who underwent a first HSCT from a serologically HLA-A, -B, and -DR matched unrelated donors between 1993 and 2011, and who had full HLA-A, -B, -C, and -DRB1 allele data, were included in this study. Bone marrow was exclusively used as a stem cell source. Clinical data for

Table 2
Multivariate Analysis to Evaluate the Impact of Single HLA Allele Mismatches on the Incidence of Grade III to IV Acute GVHD Stratified according to the Transplantation Time Period

Year	Factor		Hazard Ratio	*P* Value
1993-2001				
	Donor age		1.02 (1.00-1.03)	.082
	Donor sex	Female	1.00	
		Male	1.65 (1.05-2.60)	.031
	Female to male transplantation	No	1.00	
		Yes	1.52 (.91-2.55)	.11
	Disease	AML	1.00	
		ALL	1.15 (.79-1.68)	.47
		CML	1.62 (1.11-2.36)	.012
		MDS	.65 (.32-1.35)	.25
	Disease risk	Low	1.00	
		High	1.30 (.93-1.83)	.13
		Others	.80 (.23-2.85)	.74
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.83 (.61-1.14)	.25
	HLA	Low-risk mismatch	1.00	
		Match	.89 (.65-1.21)	.44
		High-risk mismatch	2.74 (1.73-4.32)	<.0001
2002-2007				
	Donor age		1.03 (1.01-1.05)	.0028
	Donor sex	Female	1.00	
		Male	1.50 (.96-2.33)	.076
	Female to male transplantation	No	1.00	
		Yes	1.53 (.89-2.64)	.13
	Disease	AML	1.00	
		ALL	1.36 (.95-1.96)	.094
		CML	1.27 (.74-2.20)	.38
		MDS	1.25 (.77-2.02)	.37
	Disease risk	Low	1.00	
		High	1.76 (1.25-2.48)	.0011
		Others	1.65 (.82-3.34)	.16
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.86 (.63-1.19)	.37
	HLA	Low-risk mismatch	1.00	
		Match	.64 (.46-.89)	.008
		High-risk mismatch	1.06 (.58-1.93)	.85
2008-2011				
	Donor age		1.03 (1.01-1.06)	.0016
	Donor sex	Female	1.00	
		Male	1.28 (.78-2.12)	.33
	Female to male transplantation	No	1.00	
		Yes	.98 (.52-1.88)	.96
	Disease	AML	1.00	
		ALL	1.18 (.80-1.74)	.42
		CML	1.53 (.69-3.37)	.3
		MDS	.66 (.36-1.20)	.17
	Disease risk	Low	1.00	
		High	1.53 (1.08-2.17)	.018
		Others	NA (NA-NA)	NA
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.82 (.55-1.24)	.34
	HLA	Low-risk mismatch	1.00	
		Match	.56 (.39-.80)	.0014
		High-risk mismatch	.40 (.10-1.64)	.21

AML indicates acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus.

these patients were obtained from the TRUMP [8]. We excluded patients who lacked data on survival status, those with more than 1 allele or antigen mismatch, those who received a reduced-intensity conditioning regimen, and those who received ex vivo or in vivo T cell depletion, such as antithymocyte globulin or alemtuzumab. Finally, 3718 patients were included in the main part of this study. As a post hoc analysis, 415 patients with 2 LR-MMs and 66 patients with 2 allele mismatches including at least 1 HR-MM were added to compare the impact of 1 HR-MM and 2 LR-MMs and to analyze the statistical interaction between HR-MM and the presence of an additional allele mismatch. The study was approved by the data management committee of TRUMP and by the institutional review board of Saitama Medical Center, Jichi Medical University.

Histocompatibility

Histocompatibility data for serological and genetic typing for the HLA-A, HLA-B, HLA-C, and HLA-DR loci were obtained from the TRUMP database, which includes HLA allele data determined retrospectively by the Japan Marrow Donor Program using frozen samples [7,9]. In this study, the following donor-recipient HLA-mismatch combinations were regarded as HR-MMs: A*02:06-A*02:01, A*02:06-A*02:07, A*26:02-A*26:01, A*26:03-A*26:01, B*15:01-B*15:07, C*03:03-C*15:02, C*03:04-C*08:01, C*04:01-C*03:03, C*08:01-C*03:03, C*14:02-C*03:04, C*15:02-C*03:04, C*15:02-C*14:02, DR*04:05-DR*04:03, and DR*14:03-DR*-DR1401, as we did not have enough data on HLA-DP and -DQ [7]. In HR-MM pairs, the donor and the recipient must have the HLA allele as shown above, and at the same time, these donor and recipient HLA alleles should not be shared by the recipient and the donor, respectively. For example, if the donor has HLA-A*02:06/02:06 and the recipient has HLA-A*02:01/02:06, this pair was not regarded as HR-MM pair, as the donor's HLA-A*02:06 was shared by the recipient. Other HLA mismatch pairs were regarded as LR-MM pairs. Only the HLA-C mismatch group included HLA mismatch at a serological (antigen) level.

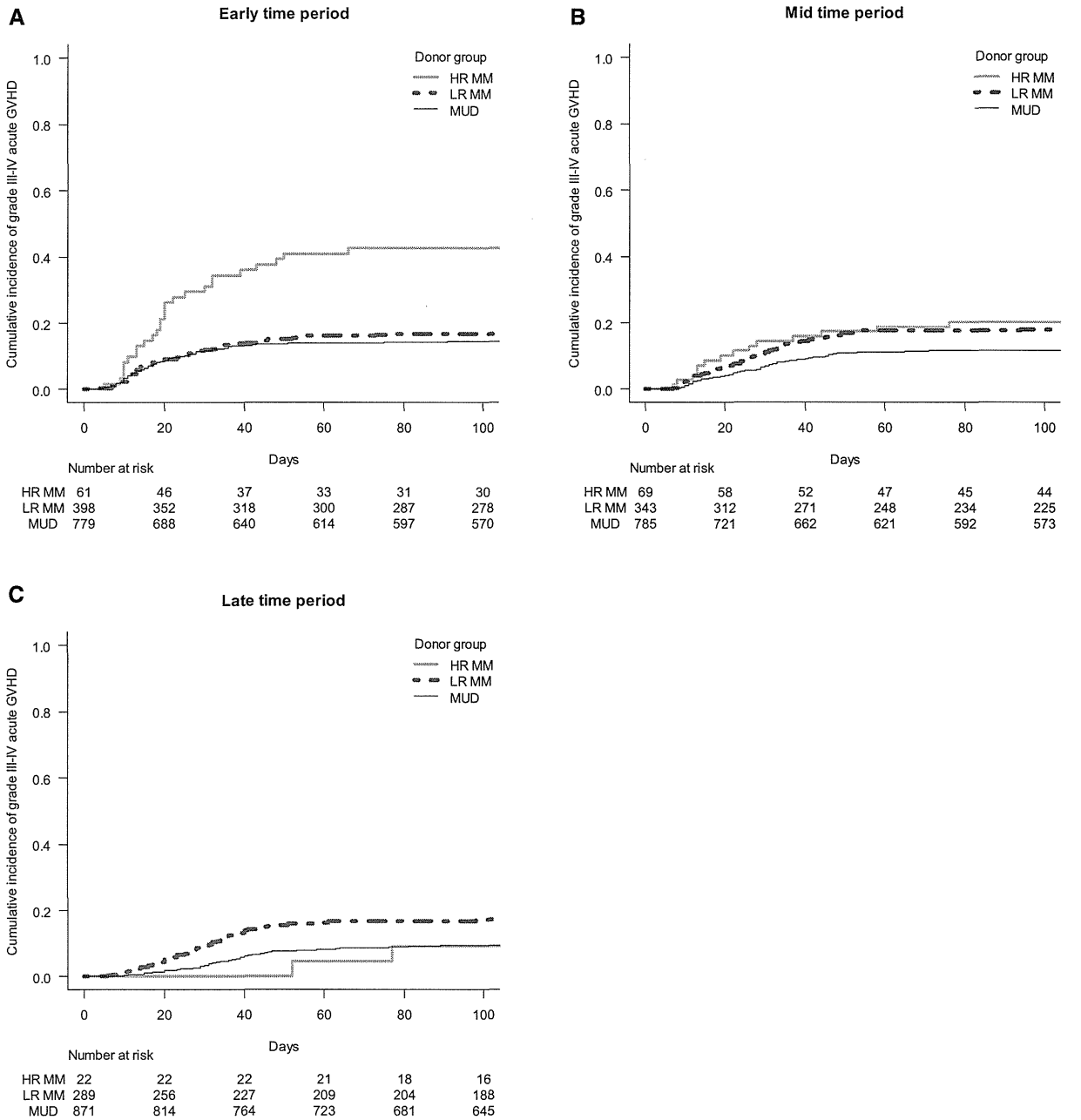

Figure 1. The cumulative incidence of grade III to IV acute GVHD grouped according to the HLA mismatch between the donor and recipient in the early (A), mid (B), and late time periods (C). HR-MM indicates high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

Statistical Analyses

We divided the patients into 3 groups according to the time period when HSCT was performed to evaluate whether the impact of HR-MM changed over time periods: the early, mid, and late groups included HSCT performed from 1993 through 2001, 2002 through 2007, and 2008 through 2011, respectively. The break points among groups were determined to make the number of patients in each group equivalent (n = 1278, 1236, and 1204, respectively). To avoid making misleading conclusions by arbitrary grouping, we confirmed that there was a statistically significant interaction between the presence of HR-MMs and transplantation year as a continuous variable, both for overall survival ($P = .0098$) and the incidence of grade III to IV acute GVHD ($P < .001$). The following analyses were performed separately in each group. However, in post hoc analyses to evaluate the impact of HR-MMs at each locus and to compare 1 HR-MM and 2 LR-MMs, the mid and late groups were combined to increase the statistical power, after confirming that similar results were obtained in the 2 groups.

The primary endpoint was the incidence of grade III to IV acute GVHD. Overall survival was evaluated as a secondary endpoint. The chi-square test or Fisher exact test was used to compare categorical variables and Student *t*-test or an analysis of variance test was used for continuous variables to evaluate the homogeneity of background characteristics of the HR-MM, LR-MM, and HLA-matched (MUD) groups. *P* values were adjusted using the Bonferroni's method and Tukey's method for multiple comparisons between each pair. Overall survival was estimated according to the Kaplan-Meier method, and compared among groups with the log-rank test. The incidence of acute GVHD was calculated treating death without GVHD as a competing event, and it was compared using Gray's test [10].

The impact of HR-MMs was evaluated using multivariate models: the Cox proportional hazards model was used for overall survival and Fine and Gray's proportional hazards model was used for acute GVHD [11]. The LR-MM group was regarded as the reference group. Potential confounding factors that were considered in these analyses included recipient/donor age, recipient/donor sex, sex mismatch, ABO major/minor mismatch, the use of

Table 3
Multivariate Analysis to Evaluate the Impact of Single High-Risk Allele Mismatches on Overall Survival Stratified According to the Transplantation Time Period

Year	Factor		Hazard Ratio	*P* Value
1993-2001				
	Age		1.02 (1.01-1.03)	<.0001
	Sex	Female	1.00	
		Male	1.06 (.90-1.23)	.51
	Disease	AML	1.00	
		ALL	1.20 (.99-1.45)	.065
		CML	.89 (.72-1.10)	.29
		MDS	.61 (.45-.83)	.0015
	Disease risk	Low	1.00	
		High	2.72 (2.30-3.23)	<.0001
		Others	2.03 (1.27-3.23)	.0029
	ABO major mismatch	Absent	1.00	
		Present	1.25 (1.06-1.47)	.0092
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.85 (.72-1.00)	.049
	HLA	Low-risk mismatch	1.00	
		Match	.86 (.73-1.01)	.063
		High-risk mismatch	1.46 (1.06-2.01)	.019
2002-2007				
	Age		1.01 (1.00-1.02)	.0025
	Sex	Female	1.00	
		Male	1.20 (1.02-1.41)	.0027
	Disease	AML	1.00	
		ALL	1.16 (.96-1.39)	.13
		CML	.84 (.62-1.12)	.23
		MDS	.56 (.43-.73)	<.0001
	Disease risk	Low	1.00	
		High	2.87 (2.41-3.40)	<.0001
		Others	2.23 (1.58-3.15)	<.0001
	ABO major mismatch	Absent	1.00	
		Present	.97 (.81-1.16)	.77
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.97 (.83-1.15)	.76
	HLA	Low-risk mismatch	1.00	
		Match	.83 (.69-.98)	.032
		High-risk mismatch	1.06 (.75-1.48)	.75
2008-2011				
	Age		1.02 (1.01-1.03)	<.0001
	Sex	Female	1.00	
		Male	1.08 (.89-1.31)	.42
	Disease	AML	1.00	
		ALL	.97 (.76-1.25)	.83
		CML	.97 (.57-1.64)	.9
		MDS	.65 (.48-.87)	.004
	Disease risk	Low	1.00	
		High	2.73 (2.23-3.35)	<.0001
		Others	NA (NA-NA)	NA
	ABO major mismatch	Absent	1.00	
		Present	1.14 (.92-1.41)	.22
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.95 (.75-1.21)	.69
	HLA	Low-risk mismatch	1.00	
		Match	.86 (.69-1.06)	.15
		High-risk mismatch	.82 (.42-1.62)	.58

AML indicates acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus.

total body irradiation in the conditioning regimen, cell dose in the bone marrow graft, the use of cyclosporine or tacrolimus as GVHD prophylaxis, background disease, and disease risk. Acute leukemia in first or second remission, CML in first or second chronic phase, CML in accelerated phase, and myelodysplastic syndrome of refractory anemia or refractory anemia with excess blasts were considered low-risk diseases, and other conditions were considered high-risk diseases. All of these potential confounding factors were included in the multivariate analyses and then deleted in a stepwise fashion from the model to exclude factors with a *P* value of .05 or higher. Finally, HLA mismatch was added to the model. Different multivariate models were compared using the likelihood ratio test. The quantity of interest was the deviance difference between the 2 models, under the null hypothesis that 2 models fit the data equally well and the deviance difference has an approximate chi-square distribution with degrees of freedom equal to the difference in the number of independent variables between the compared models.

All *P* values were 2 sided and *P* values of .05 or less were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University) [12], which is a graphical user interface for R (The R Foundation for Statistical Computing). More precisely, it is a modified version of R commander that was designed to add statistical functions frequently used in biostatistics.

RESULTS

Patients

The patient characteristics are summarized in Table 1. HR-MMs were observed in 64 of 1278, 71 of 1236, and 22 of 1204 donor-recipient pairs in the early, mid, and late time periods, respectively. On the other hand, 412, 351, and 294 pairs had LR-MMs, respectively. With regard to the

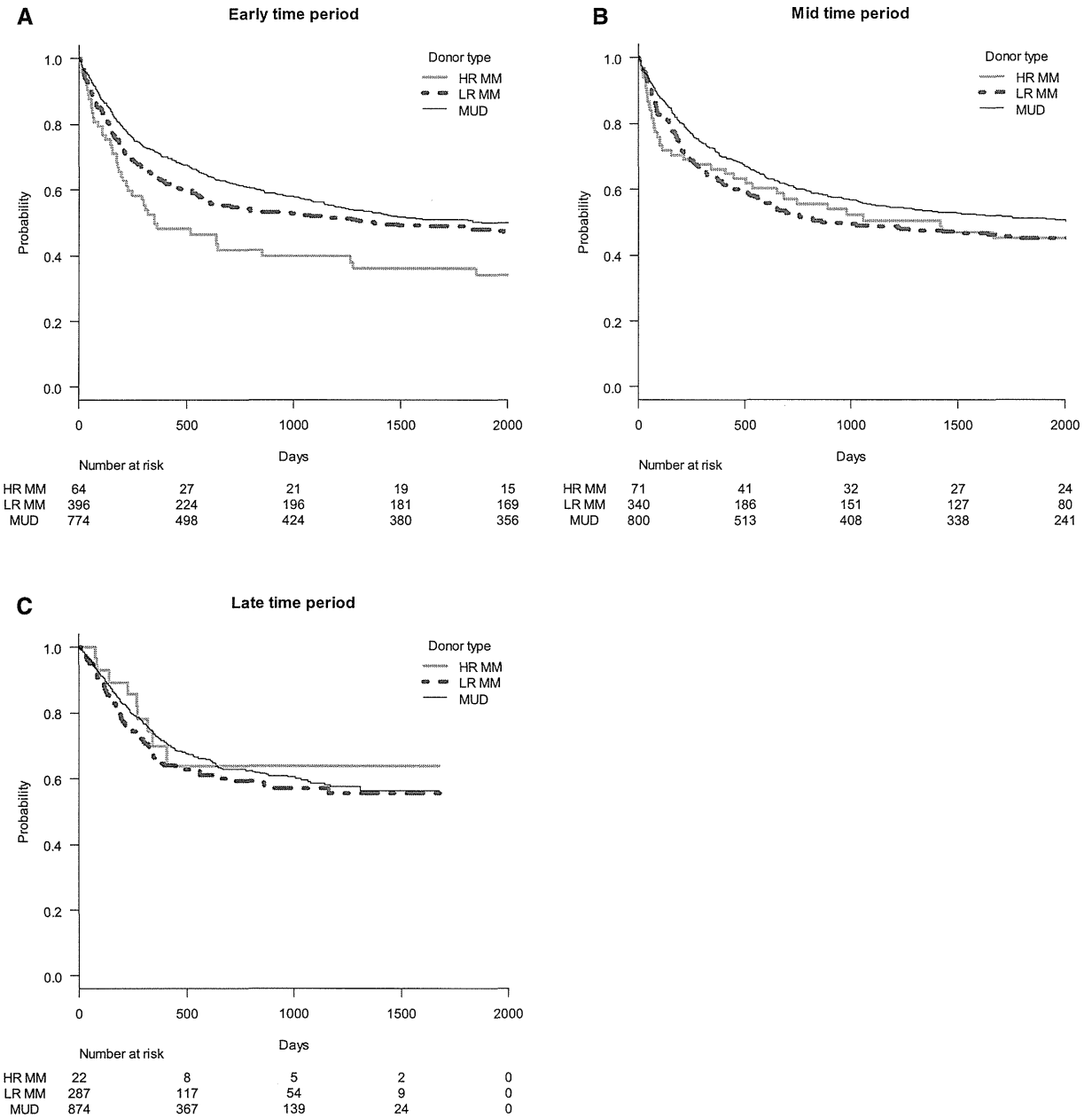

Figure 2. Overall survival grouped according to the HLA mismatch between the donor and recipient in the early (A), mid (B), and late time periods (C). The survival curves were adjusted for other significant factors by the mean of covariates method, in which average values of covariates are entered into the Cox proportional hazards model. HR-MM, high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

differences among transplantation time periods, the numbers of LR-MMs and HR-MMs decreased in the late time periods, ie, after the introduction of routine typing for HLA-C and the publication of a paper about HR-MMs [7]. The proportion of HSCTs for CML also dramatically decreased over time periods (30.7%, 10.4%, and 3.6% in the early, mid, and late periods, respectively). With regard to the difference among HLA mismatch groups, the proportion of patients with high-risk underlying disease in the MUD group (29.9%) was significantly lower than those in the HR-MM (37.6%) and LR-MM groups (34.4%). In addition, the proportion of HSCTs for CML was significantly higher in the HR-MM group in the early time period (29.6%, 30.3%, and 46.9% in the MUD, LR-MM, and HR-MM groups, respectively).

Incidence of Grade III to IV Acute GVHD

To adjust the impact of HLA mismatch for possible confounding factors, we identified the following independently significant factors for the incidence of grade III to IV acute GVHD: donor age, donor sex, sex mismatch, disease, disease risk, and GVHD prophylaxis. After we adjusted for these factors, we confirmed that the incidence of grade III to IV acute GVHD in the HR-MM group was significantly higher than that in the LR-MM group (hazard ratio [HR], 2.74; 95% confidence interval [CI], 1.73 to 4.32; $P < .0001$) in the early time period, whereas the difference between the MUD and LR-MM groups was not significant (HR, .89; 95% CI, .65 to 1.21; $P = .44$) (Table 2, Figure 1). On the other hand, in the mid and late time periods, the difference in the incidence of

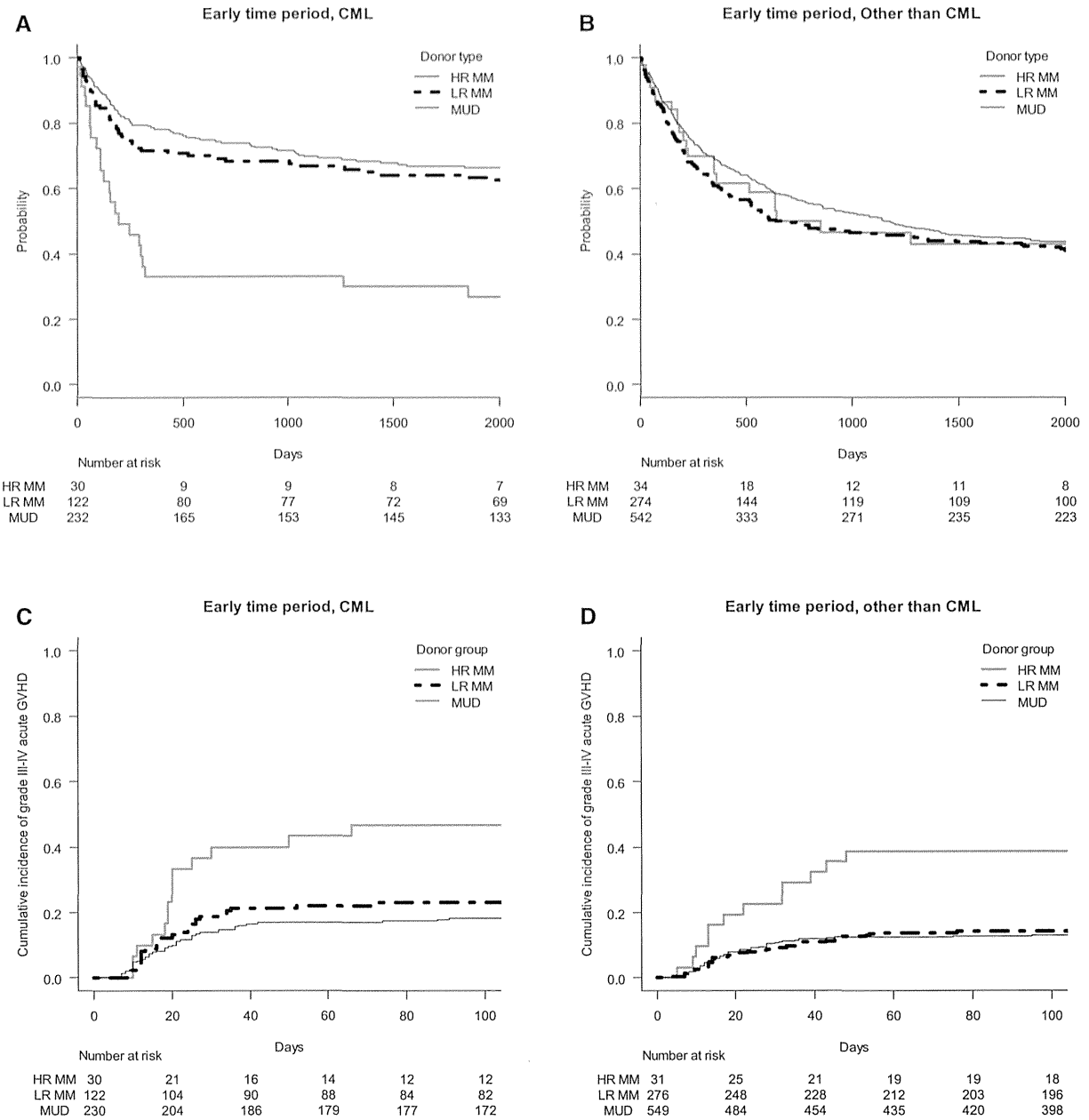

Figure 3. Adjusted overall survival (A,B) and the cumulative incidence of grade III to IV acute GVHD (C,D) grouped according to the underlying disease in the early time period. CML, chronic myelogenous leukemia; HR-MM, high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

grade III to IV acute GVHD between the HR-MM and LR-MM groups was not statistically significant (HR, 1.06; 95% CI, .58 to 1.93; $P = .85$ and HR, .40; 95% CI; .10 to 1.64; $P = .21$, respectively). The presence of LR-MM significantly adversely affected the incidence of grade III to IV acute GVHD in the mid and late periods (HR, .64; 95% CI, .46 to .89; $P = .008$ and HR, .56; 95% CI, .39 to .80; $P = .0014$, respectively, for the MUD group).

Similarly, the presence of HR-MM significantly affected the incidence of grade II to IV acute GVHD compared with LR-MM only in the early time period (HR, 1.53; 95% CI, 1.05 to 2.24; $P = .028$), and not in the mid and late periods (HR, .92; 95% CI, .61 to 1.37; $P = .67$ and HR, .79; 95% CI, .40 to 1.58; $P = .51$, respectively).

Overall Survival

After adjusting for recipient age, recipient sex, presence of ABO-major mismatch, disease, disease risk, and GVHD prophylaxis, we again confirmed that survival in the HR-MM group was significantly inferior to that in the LR-MM group (HR, 1.46; 95% CI, 1.06 to 2.01; $P = .019$) in the early time period, whereas there was no significant difference between the MUD and LR-MM groups (HR, .86; 95% CI, .73 to 1.01; $P = .063$) (Table 3). On the other hand, the difference in survival between the HR-MM and LR-MM groups was not statistically significant in the mid and late time periods (HR, 1.06; 95% CI, .75 to 1.48; $P = .75$ and HR, .82; 95% CI, .42 to 1.62; $P = .58$, respectively). The difference in survival between the MUD and LR-MM groups was consistent among

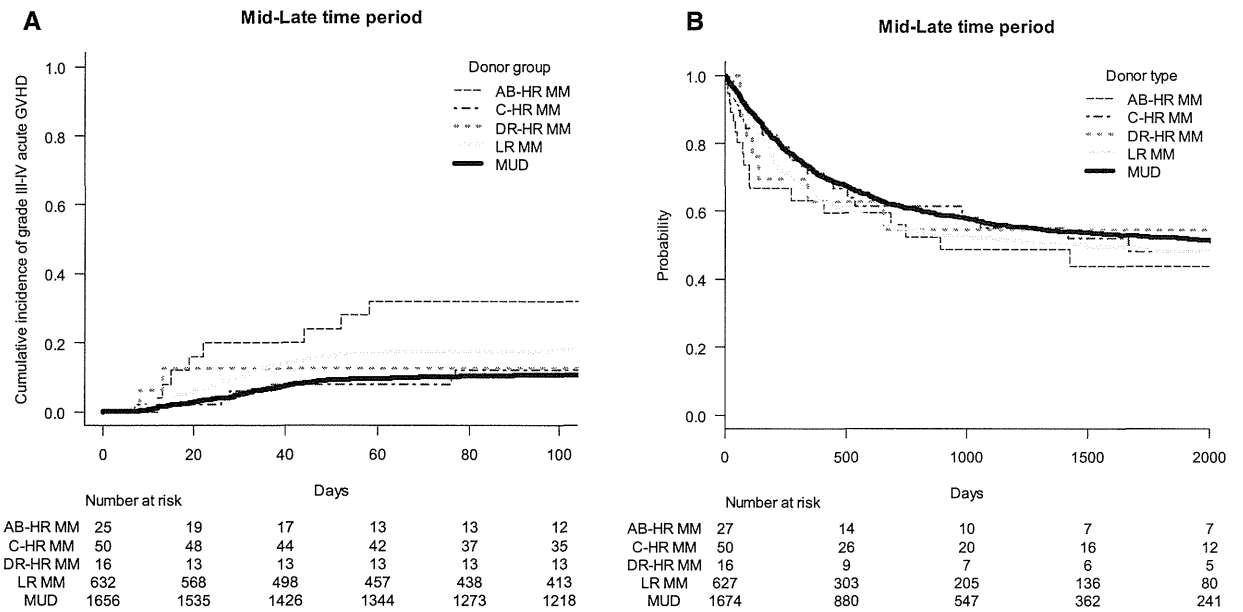

Figure 4. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch loci between the donor and recipient in the mid or late time period. AB-HR MM, high-risk mismatch at the HLA-A or -B locus; C-HR MM, high-risk mismatch at the HLA-C locus; DR-HR MM, high-risk mismatch at the DRB1 locus; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

the 3 time periods but statistically significant only in the mid period (HR, .83; 95% CI, .69 to .98; $P = .032$). Figure 2 shows the overall survival curves grouped according to the HLA-mismatch groups in each time period, adjusted for other significant factors by the mean of covariates method.

Disease-specific Effects of HR-MM in the Early Period

The number of patients with CML was significantly higher in the early period than in the mid and late periods. Therefore, we evaluated the disease-specific impact of HR-MM in the early period. As shown in Figures 3A and B, the presence of HR-MM had an adverse impact on overall survival only in patients with CML, although HR-MM showed a similar adverse impact on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C, D). Of the 24 CML patients who died after HSCT with HR-MM, 23 died without relapse of CML, and 10 of these patients died without grade III to IV acute GVHD.

Impact of HR-MM at Each Locus

To evaluate the impact of HR-MM at each locus in the mid and early periods, we combined the 2 periods together to

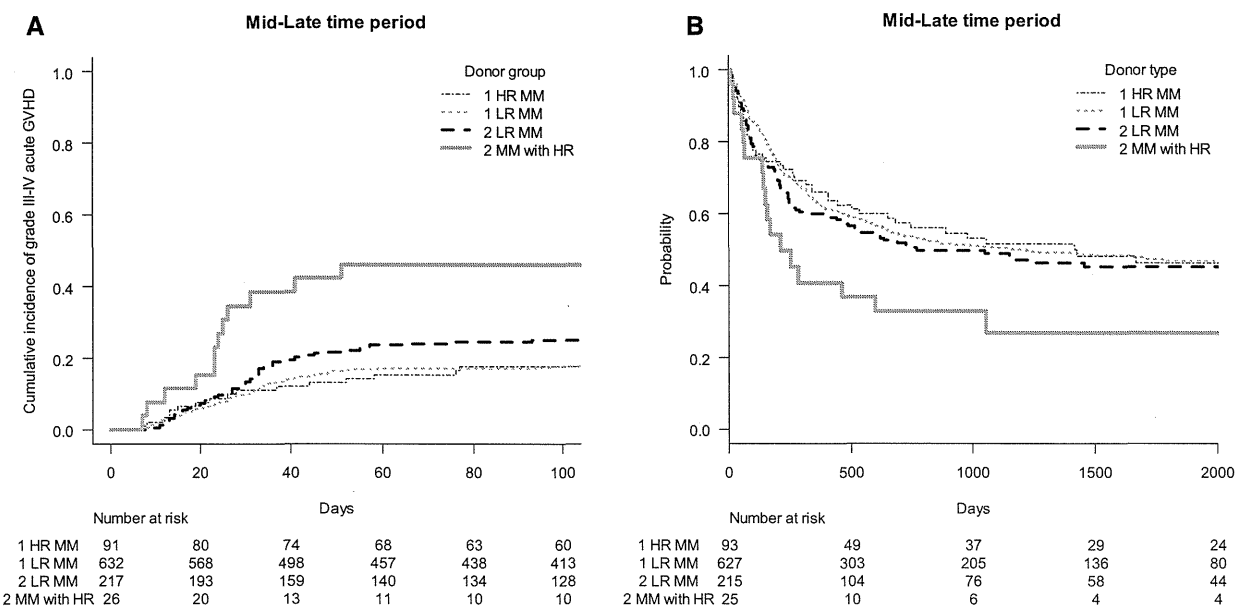

Figure 5. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch between the donor and recipient in the mid or late time period. 1HR-MM, 1 high-risk mismatch; 1LR-MM, 1 low-risk mismatch; 2LR-MM, 2 low-risk mismatches; 2MM with HR, 2 allele mismatches including at least 1 HR-MM.

increase statistical power because the impact of HR-MM on acute GVHD and survival tended to be similar in these 2 time periods. The presence of HR-MMs at the HLA-A/B (HLA-A or -B), HLA-C, and HLA-DRB1 loci was not associated with significantly different survival compared with the LR-MM group (HR, 1.23; 95% CI, .76 to 1.98; $P = .41$; HR, .96; 95% CI, .65 to 1.44; $P = .86$; and HR, .95; 95% CI, .45 to 2.02; $P = .89$, respectively. Figure 4A). However, the incidence of grade III to IV acute GVHD was higher in patients who had HR-MM at the HLA-A/B locus than in those with LR-MM, although this difference was not statistically significant (HR, 1.78; 95% CI, .86 to 3.66; $P = .12$; HR, .63; 95% CI, .28 to 1.41; $P = .26$; and HR, .69; 95% CI, .15 to 3.12; $P = .63$ for HLA-A/B, HLA-C, and HLA-DRB1, respectively.) (Figure 4B).

Comparison of One HR-MM and Two LR-MMs

To evaluate whether a donor with 1 HR-MM or a donor with 2 LR-MMs should be preferred, we added patients with 2 LR-MMs and those with 2 allele mismatches including at least 1 HR-MM to the dataset, and we compared the outcome of HSCT from these donors with that of HSCT from a donor with 1 LR-MM as a reference in the combined mid and late periods.

The presence of 2 LR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD (HR, 1.44; 95% CI, 1.04 to 2.00; $P = .030$), but the impact of 1 HR-MM was not statistically significant (HR, .94; 95% CI, .56 to 1.59; $P = .83$) (Figure 5A). However, the impact of 2 LR-MMs was not associated with inferior survival. The HR for survival of 1 HR-MM and 2 LR-MMs were 1.05 (95% CI, .78 to 1.42; $P = .75$) and 1.12 (95% CI, .90 to 1.39; $P = .33$), respectively (Figure 5B).

On the other hand, the presence of 2 allele mismatches including at least 1 HR-MM was associated with an extremely poor outcome; HR, 3.61 (95% CI, 1.96 to 6.66; $P < .001$) for grade III to IV acute GVHD and HR, 2.02 (95% CI, 1.25 to 3.26; $P = .0040$) for overall survival. These results suggested that the impact of HR-MM may change according to the presence or absence of an additional allele mismatch. In fact, there was a statistically significant interaction between the presence of HR-MM and the presence of an additional allele mismatch ($P = .020$). The likelihood ratio test revealed that the prognostic value of Fine and Gray's proportional hazards model for acute GVHD was significantly improved by adding the interaction term to the model ($P = .024$).

DISCUSSION

In this study, we reevaluated the clinical impact of HR-MMs in unrelated HSCT. We confirmed that the presence of HR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD and significantly inferior survival in the early transplantation time period. However, in the mid and late periods, ie, after 2002, there was no statistically significant difference in overall survival or the incidence of grade III to IV acute GVHD between patients with HR-MMs and those with LR-MMs. The methods used for the statistical analyses were somewhat different than those in a previous study, but this is not the major reason for the different results, as the significant impact of HR-MMs on survival and acute GVHD was reproduced in the early time period. Another possible explanation is a bias caused by the availability of information about HR-MMs. After the publication of a paper that reported the importance of HR-MM, physicians may have tended to intensify prophylaxis against GVHD in unrelated HSCT with HR-MMs, and, thereby, the impact of HR-MMs might have become less significant. However, this is not the case because the impact of HR-MMs was already not apparent in the mid time period, before the paper was published. We also considered that the difference in the underlying disease might have influenced the effect of HR-MMs. The proportion of patients with CML decreased from 30.7% in the early period to 10.4% and 3.6% in the mid and late periods, respectively. Therefore, we analyzed the impact of HR-MMs grouped according to the underlying disease in the early period. The effect of HR-MMs on survival was observed only in patients with CML (Figure 3A,B). However, HR-MMs had an adverse effect on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C,D). Therefore, the different effects of HR-MMs on the incidence of grade III to IV acute GVHD among the time periods could not be explained solely by the underlying diseases. We could not clarify the reason for this different effect, but the changes in the transplantation procedure, including prophylaxis against GVHD, might have reduced the clinical impact of HR-MM. In fact, the incidence of grade III to IV acute GVHD decreased from 42.6%, 16.8%, and 14.5% in the HR-MM, LR-MM, and MUD groups, respectively, in the early time period to 17.6%, 17.7%, and 10.6% in the mid or late period. Improved survival in patients who developed severe acute GVHD might also reduce the effect of HR-MMS on survival. The 1-year survival in patients who developed grade III to IV acute GVHD improved from 32.1% in the early period to 44.4% in the mid and late time periods. This change may have resulted from the progress in supportive care, including strategies against fungal or viral infections.

Another important finding is that the impact of HR-MM was significantly enhanced by the presence of an additional allele mismatch in the mid and late time periods. This fact may be explained by a hypothesis that the HR-MM biologically increases the graft-versus-host (GVH) reaction, but the recent improvement in GVHD prophylaxis has masked its effect, if HR-MM exists as a single allele mismatch, whereas the adverse impact of HR-MM is not suppressed even by recent methods of GVHD prophylaxis when an additional allele mismatch is present. Based on these findings, interaction terms should be incorporated into the statistical model when the impact of HR-MMs is analyzed in datasets that include HSCT with multiple allele mismatches.

A major limitation of this study is the small number of patients with HR-MMs, especially in the late time period. We cannot deny the possibility that an important effect of HR-MMs might be overlooked because of the poor statistical power. The lack of a significant difference in the incidence of grade III to IV acute GVHD between unrelated HSCT with HR-MMs at the HLA-A/B locus and HSCT with LR-MM should be interpreted with caution, because of the small number of patients. Furthermore, it was impossible to evaluate the effect of each mismatch combination, as the number of patients with each mismatch combination was most often fewer than 10. HR-MMs associated with at least a 20% incidence of grade III to IV acute GVHD in the mid and late periods included A*0206-A*0201 (4 of 14), A*0206-A*0207 (3 of 4), B*1501-B*1507 (1 of 1), C*0801-C*0303 (4 of 15), and C*1402-C*0304 (1 of 5), but the number of patients in each pair was too small to draw any definitive conclusions.

When we consider the impact of HR-MMs, especially at the HLA-C locus, we should also consider the effect of a killer immunoglobulin-like receptor ligand (KIR) mismatch [13,14]. Among the 50 patients with HR-MMs at the HLA-C locus in the mid and late periods, 20 had a KIR mismatch in the GVH direction, whereas 30 did not. The incidence of grade III to IV acute GVHD was 5% and 16.7%, respectively, but this

difference was not statistically significant ($P = .24$). The incidence of grade III to IV acute GVHD in the 21 patients who had LR-MMs and a KIR mismatch in the GVH direction was 15.0%. We could not conclude that a KIR mismatch had an impact in this study because of the small number of patients with a KIR mismatch in the GVH direction.

We should note that the results of the current study are applicable to patients who receive bone marrow graft after a myeloablative conditioning regimen. The impact of HR-MMs may change according to the stem cell source or the conditioning regimen. Therefore, further analyses are required to evaluate the impact of HR-MMs in peripheral blood stem cell transplantation and reduced-intensity conditioning transplantation.

In conclusion, this retrospective study revealed that the clinical impact of HR-MMs became less significant after 2002. Although HR-MMs may have a biological impact, their effect may be controlled by recent methods for GVHD prophylaxis when they exist as a single allele mismatch. It may still be prudent to avoid a donor with HR-MMs, especially at the HLA-A or -B locus, if a donor with the other mismatch combination is available. However, in the absence of MUD or an unrelated donor with a LR-MM, a donor with a single HR-MM could be a viable option for unrelated HSCT, and it is preferred over a donor with 2 LR-MMs. In addition, we should be aware that the clinical impact of risk factors may change over time periods, and therefore, we should repeatedly confirm the validity of risk factors.

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Authorship statement: Y.K. designed the study. Y.K. and J.K. analyzed the data. Y.A., S.F., Y.M., T.I., M.T., K.O., T.F., K.M., T.M., C.K., N.K., K.I., A.S., and S.M. gathered the data. Y.K. wrote the first draft of the paper and all other authors contributed to the final version.

REFERENCES

1. 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;104:1923-1930.
2. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110:4576-4583.
3. Petersdorf EW, Anasetti C, Martin PJ, et al. Limits of HLA mismatching in unrelated hematopoietic cell transplantation. *Blood*. 2004;104:2976-2980.
4. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. *N Engl J Med*. 1998;339:1177-1185.
5. Kanda Y, Kanda J, Atsuta Y, et al. Impact of a single human leucocyte antigen (HLA) allele mismatch on the outcome of unrelated bone marrow transplantation over two time periods. A retrospective analysis of 3003 patients from the HLA Working Group of the Japan Society for Blood and Marrow Transplantation. *Br J Haematol*. 2013;161:566-577.
6. Zino E, Frumento G, Marktel S, et al. A T-cell epitope encoded by a subset of HLA-DPB1 alleles determines nonpermissive mismatches for hematologic stem cell transplantation. *Blood*. 2004;103:1417-1424.
7. Kawase T, Morishima Y, Matsuo K, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. *Blood*. 2007;110:2235-2241.
8. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol*. 2007;86:269-274.
9. 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;99:4200-4206.
10. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.
11. Fine JP, Gray RJ. A proportional hazards model for subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:456-509.
12. Kanda Y. Investigation of the freely available easy-to-use software "EZR" (Easy R) for medical statistics. *Bone Marrow Transplant*. 2013;48:452-458.
13. 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;13:315-328.
14. Leung W. Use of NK cell activity in cure by transplant. *Br J Haematol*. 2011;155:14-29.

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ORIGINAL ARTICLE

Impact of HLA allele mismatch on the clinical outcome in serologically matched related hematopoietic SCT

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In unrelated hematopoietic SCT (HSCT), HLA allele mismatch has been shown to have a significant role. To clarify the importance of HLA allele mismatch in the GVH direction in related HSCT, we retrospectively evaluated 2377 patients who received stem cells from an HLA serologically matched related donor in the GVH direction using the database of the Japan Society for Hematopoietic Cell Transplantation. The cumulative incidences of grade II–IV and grade III–IV acute GVHD in patients with an HLA allele-mismatched donor ($n = 133$, 5.6%) were significantly higher than those in patients with an HLA allele-matched donor. Multivariate analyses showed that the presence of HLA allele mismatch was associated with increased risks of grade II–IV and grade III–IV acute GVHD. In particular, HLA-B mismatch and multiple allele mismatches were associated with an increased risk of acute GVHD. The presence of HLA allele mismatch was associated with an inferior OS owing to an increased risk of non-relapse mortality (NRM). In conclusion, the presence of HLA allele mismatch in the GVH direction in related HSCT was associated with increased risks of GVHD and NRM, which led to an inferior OS. HLA allele typing is recommended in related HSCT.

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INTRODUCTION

Previous studies have shown that HLA allele mismatch significantly affects the clinical outcome after unrelated hematopoietic SCT (HSCT).[1,2] Several retrospective studies have demonstrated that the presence of HLA allele mismatch is associated with an increased risk of GVHD in unrelated HSCT.[3–5] Although the disparity of HLA molecules in HLA antigen mismatch is greater than that in HLA allele mismatch without HLA antigen mismatch, the impact of HLA mismatch on the clinical outcome was considered to be, for practical purposes, similar between antigen mismatch and allele mismatch, as reported previously.[4,6,7] Although the impact of an HLA mismatch at each locus varied among the studies, there is a consensus that an HLA mismatch at any locus, including A, B, C and DRB1, is in general associated with a poor clinical outcome.[2]

In related HSCT, the importance of HLA allele mismatch has not yet been well established, because an HLA antigen-matched sibling is in most cases an HLA allele fully matched donor. In Japan, HLA compatibility in related HSCT is usually assessed serologically or by low-resolution DNA typing at three loci, including HLA-A, -B and -DR. However, when the donor is not a sibling, such as a parent or child, the probability of HLA allele mismatch between the recipient and the donor is expected to be higher than that between siblings. Furthermore, there may also be an HLA allele mismatch with a sibling if we consider recombination and mutation. The presence of one HLA antigen mismatch has been reported to be associated with a poor overall clinical outcome in related HSCT.[8–10] Therefore, if the impact of allele mismatch is similar to that of antigen mismatch in related HSCT, as it is in unrelated HSCT, we could assume that the presence of HLA allele mismatch adversely affects the clinical outcome in related HSCT.

In this study, we assessed the impact of HLA allele mismatch on the clinical outcome in related HSCT using the database of the Japan Society for Hematopoietic Cell Transplantation (JSHCT), including patients without serological HLA mismatch in the GVH direction.

PATIENTS AND METHODS

Data collection

Data for all patients who received a first allogeneic HSCT from a serologically HLA-A, -B and -DR matched related donor in the GVH direction, irrespective of the number of mismatches in the HVG direction, between 1 January 2000 and 31 December 2011 were obtained from the Transplant Registry Unified Management Program, which includes data from the JSHCT.[11] We excluded patients who lacked data on survival status. Overall, 7089 patients satisfied the above criteria. In further analyses, we considered only 2377 patients (33.5%) for whom information

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on allele typing at the HLA-A, -B, and -DRB1 loci was available. The study was planned by the HLA working group of the JSHCT and was approved by the data management committees of TRUMP and by the institutional review board of Saitama Medical Centre, Jichi Medical University, Saitama, Japan.

Histocompatibility

Histocompatibility data for serological and genomic typing for the HLA-A, -B and -DR loci were obtained from reports obtained from the institution at which the transplantation was performed. To reflect current practice in Japan, HLA matching in related donors was assessed by serological data for HLA-A, -B, and -DR loci. When the recipient's antigens or alleles were not shared by the donor, this was considered an HLA mismatch in the GVH direction; when the donor's antigens or alleles were not shared by the recipient, this was considered a mismatch in the host-versus-graft (HVG) direction.

End points and statistical analyses

The primary end point was the cumulative incidence of acute GVHD. Secondary end points included the cumulative incidences of neutrophil engraftment and non-relapse mortality (NRM) and the probability of OS. The physicians who performed transplantation at each center diagnosed and graded acute GVHD according to the standard criteria.[12]

A descriptive statistical analysis was performed to assess the patients' characteristics. Medians and ranges are provided for continuous variables, and the percentages are shown for categorical variables. Patient's characteristics were compared by using the Chi-squared test or the Fisher's exact test for categorical variables. The probability of OS was calculated by the Kaplan–Meier method. A Cox proportional-hazards regression model was used to analyze OS. The cumulative incidences of NRM, GVHD and relapse were evaluated using the model of Fine and Grey[13] for univariate and multivariate analyses. In the competing risk models for GVHD, relapse and death before these events were defined as competing risks. In the competing risk models for NRM, relapse was defined as a competing risk. Factors that were associated with a two-sided *P*-value of < 0.10 in the univariate analysis were included in a multivariate analysis. We used a backward stepwise selection algorithm and retained only statistically significant variables in the final model. A two-sided *P*-value of < 0.05 was considered statistically significant. The variables evaluated in these analyses were as follows: sex mismatch (female to male vs others), patient's age at the time of HSCT (age $\geqslant 50$ years vs age < 50 years), disease risk (standard risk vs high risk), stem cell source (BM vs PBSC), relation to donor (sibling or others), ABO mismatch, use of *in vivo* T-cell depletion, performance status (0–1 vs 2–4), intensity of the conditioning regimen (myeloablative vs reduced intensity), GVHD prophylaxis (CYA based vs tacrolimus based), year of transplant ($\geqslant$2007 vs $<$ 2007) and HLA disparity as assessed by allele typing of HLA A, B and DRB1. Standard risk was defined as the first or second CR of acute leukemia, the first or second chronic phase of chronic myeloid leukemia, myelodysplastic syndrome refractory anemia or refractory cytopenia with multilineage dysplasia, malignant lymphoma in CR or PR or non-malignant disease. High risk was defined as some other status of malignancy. All statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University, Saitama, Japan; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0).[14]

RESULTS

Patient characteristics

The patient characteristics are summarized in Table 1. The median age was 40 years (range, 0–74). Compared with recipients with an HLA allele-matched donor (Match group, $n = 2244$), recipients with an HLA allele-mismatched donor (Mismatch group, $n = 133$) were more likely to have a poor performance status, to receive a transplantation from a non-sibling donor, to receive a transplantation at an earlier time period, to receive tacrolimus for GVHD prophylaxis and to receive an *in vivo* T-cell depletion (Table 1). More patients in the Mismatch group received a transplant from a donor with an HLA mismatch in the HVG direction. In the Match group, the number of antigen mismatches in the HVG direction

Table 1. Patient characteristics

Variable	*HLA allele match in the GVH direction* n = *2244*, n *(%)*	*HLA allele mismatch in the GVH direction* n = *133*, n *(%)*	*P-value*
Age at transplantation			
Median, years (range)	40 (0–74)	36 (0–69)	0.10
$\geqslant 50$	1491 (66.4%)	93 (69.9%)	0.46
< 50	753 (33.6%)	40 (30.1%)	
Sex combination of donors and recipients			
Female to male	608 (27.1%)	34 (25.6%)	0.60
Other combinations	1625 (72.4%)	98 (73.7%)	
Missing	11 (0.5%)	1 (0.8%)	
Performance status			
0–1	1967 (87.7%)	101 (75.9%)	< 0.001
2–4	232 (10.3%)	22 (16.5%)	
Missing	45 (2.0%)	10 (7.5%)	
Disease			
AML	813 (36.2%)	38 (28.6%)	0.35
ALL	468 (20.9%)	28 (21.1%)	
MDS	247 (11.0%)	12 (9.0%)	
CML	74 (3.3%)	7 (5.3%)	
Lymphoma	340 (15.2%)	26 (19.5%)	
Non-malignant disease	247 (11.0%)	17 (12.8%)	
Others	55 (2.5%)	5 (3.8%)	
Disease risk			
Standard	1325 (59.0%)	66 (49.6%)	0.083
High	906 (40.4%)	66 (49.6%)	
Missing	13 (0.6%)	1 (0.8%)	
Relation between donor and recipient			
Sibling	2048 (91.3%)	64 (48.1%)	< 0.001
Parent/child	185 (8.2%)	65 (48.9%)	
Others[a]	11 (0.5%)	4 (3.0%)	
Source of stem cells			
BM	1162 (51.8%)	65 (48.9%)	0.57
PBSC	1082 (48.2%)	68 (51.1%)	
HLA compatibility in the GVH direction[b]			
Matched	2244 (100%)	0 (0%)	< 0.001
One allele mismatch	0 (0%)	116 (87.2%)	
HLA-A		32	
HLA-B		18	
HLA-DRB1		66	
$\geqslant$ Two allele mismatch	0 (0%)	17 (12.8%)	
HLA compability in the HVG direction[b]			
Matched	2164 (96.4%)	75 (56.4%)	< 0.001
One antigen mismatch	46 (2.0%)	44 (33.1%)	
$\geqslant$ Two antigen mismatch	34 (1.5%)	14 (10.5%)	
Conditioning regimen			
Myeloablative	1426 (63.5%)	84 (63.2%)	0.92
Reduced intensity	761 (33.9%)	43 (32.3%)	
Missing	57 (2.5%)	6 (4.5%)	
GVHD prophylaxis			
CYA based	1891 (84.3%)	47 (35.3%)	< 0.001
Tacrolimus based	285 (12.7%)	79 (59.4%)	
Missing	68 (3.0%)	7 (5.3%)	
In vivo T-cell depletion			
Yes	154 (6.9%)	25 (18.8%)	< 0.001
No	2090 (93.1%)	108 (81.2%)	
Year of transplant			
2000–2006	522 (23.3%)	49 (36.8%)	< 0.001
2007–2011	1722 (76.7%)	84 (63.2%)	

Abbreviations: HVG = host-versus-graft; MDS = myelodysplastic syndrome. [a]Others included half-sibling ($n = 4$), aunt ($n = 3$), cousin ($n = 2$), nephew ($n = 1$) and grandchild in the Match group and half-sibling ($n = 1$), cousin ($n = 2$) and unknown ($n = 1$) in the Mismatch group. [b]HLA compatibility was defined according to the HLA-A, -B and -DR loci.

was 0 in 96.4%, 1 in 2.0%, 2 in 1.0% and 3 in 0.5%. In the Mismatch group, the number of antigen mismatches in the HVG direction was 0 in 56.3%, 1 in 33.1%, 2 in 7.5% and 3 in 3.0%. Information on HLA-C allele mismatch was available in only 1152 of 2377 (48.5%).

GVHD

The cumulative incidences of grade II–IV acute GVHD were 29.5% (95% confidence interval (CI) 27.6–31.4%) in the Match group and 40.6% (95% CI 32.2–48.8%) in the Mismatch group ($P=0.0018$, Figure 1a). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an increased risk of grade II–IV acute GVHD (hazard ratio (HR) 1.77, 95% CI 1.31–2.38, $P=0.0002$, Table 2). An increase in the number of HLA mismatches was associated with a statistically significant increase in the risk of grade II–IV acute GVHD. The cumulative incidences of grade II–IV acute GVHD were 38.8% (95% CI 29.9–47.6%) and 52.9% (95% CI 26.5–73.8%) in patients with one allele mismatch and multiple allele mismatches, respectively ($P=0.0020$, Figure 1b). Compared with the Match group, both the one allele-mismatched and multiple allele-mismatched cohorts were associated with an increased risk of grade II–IV acute GVHD in multivariate analyses (one allele mismatch: HR 1.61, 95% CI 1.17–2.22, $P=0.0035$; multiple allele mismatches: HR 3.52, 95% CI 1.64–7.59, $P=0.0013$). We also assessed the impact of each locus excluding patients with multiple allele mismatches. The cumulative incidences of grade II–IV acute GVHD were 25.0% (95% CI 11.6–41.0%) in HLA-A mismatch, 50.0% (24.8–70.9%) in HLA-B mismatch and 42.4% (30.3–54.0%) in HLA-DRB1 mismatch (Figure 1c). In a multivariate analysis, the presence of HLA-B or -DRB1 mismatch was associated with an increased risk of grade II–IV acute GVHD (HLA-A: HR 0.86, 95% CI 0.40–1.84, $P=0.69$; HLA-B: HR 2.33, 95% CI 1.18–4.63, $P=0.015$; HLA-DRB1: HR 1.83, 95% CI 1.22–2.72, $P=0.0033$).

The cumulative incidences of grade III–IV acute GVHD were 9.5% (95% CI 8.3–10.8%) in the Match group and 21.8% (95% CI 15.2–29.2%) in the Mismatch group ($P<0.0001$, Figure 1d). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an increased risk of grade III–IV acute GVHD (HR 2.39, 95% CI 1.60–3.58, $P<0.0001$, Table 2). Other factors that were associated with an increased risk of grade III–IV acute GVHD were use of PBSC and high disease risk. An increase in the number of HLA mismatches was associated with a significantly increased risk of grade III–IV acute GVHD. The cumulative incidences of grade III–IV acute GVHD were 19.8% (95% CI 13.1–27.6%) and 35.3% (95% CI 13.8–57.8%) in patients with one allele mismatch and multiple allele mismatches, respectively ($P<0.0001$, Figure 1e). Compared with the Match group, both the one allele mismatch and multiple allele mismatched cohorts were associated with an increased risk of grade III–IV acute GVHD in multivariate analyses (one allele

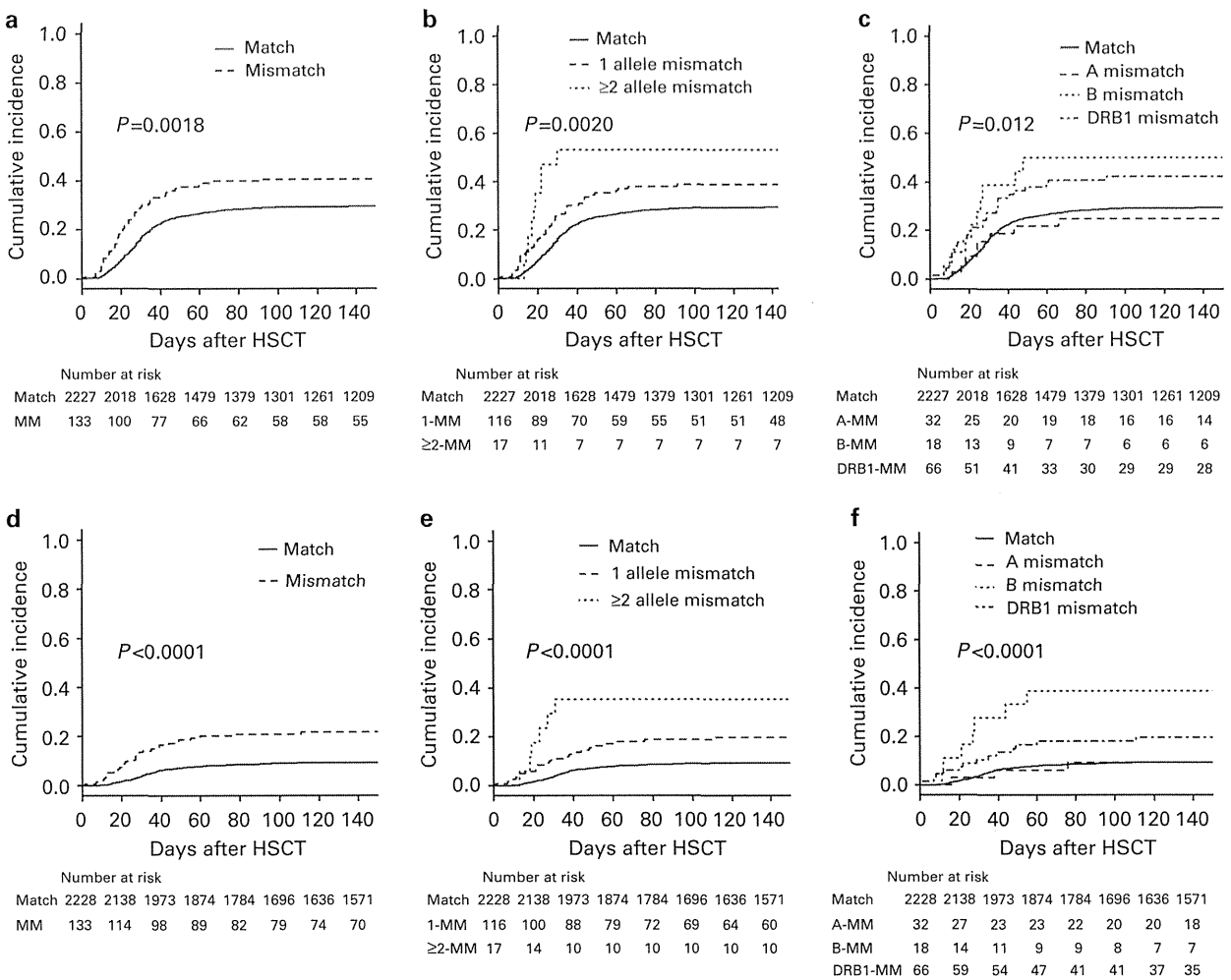

Figure 1. Cumulative incidence of acute GVHD. Cumulative incidences of grade II–IV (**a**–**c**) and grade III–IV (**d**–**f**) acute GVHD grouped according to (**a**, **d**) allele mismatch, (**b**, **e**) the number of allele mismatches and (**c**, **f**) locus of allele mismatches.

Table 2. Multivariate analysis

Outcomes and significant factors	*HR*	*95% CI*	*P-value*
Grade II–IV acute GVHD			
Use of *in vivo* TCD (vs no *in vivo* TCD)	0.58	0.39–0.85	0.0059
Age ⩾ 50 years (vs age < 50 years)	1.19	1.01–1.41	0.039
Reduced intensity (vs myeloablative)	0.78	0.66–0.92	0.0041
PBSC (vs BM)	1.32	1.13–1.53	0.0005
Allele mismatch in the GVH direction	1.77	1.31–2.38	0.0002
Grade III–IV acute GVHD			
PBSC (vs BM)	1.85	1.41–2.44	< 0.0001
Disease risk, high (vs standard)	1.59	1.22–2.08	0.0001
Allele mismatch in the GVH direction	2.39	1.60–3.58	< 0.0001
NRM			
Age ⩾ 50 years (vs age < 50 years)	1.93	1.52–2.46	< 0.0001
PBSC (vs BM)	1.52	1.19–1.94	< 0.0001
Disease risk, high (vs standard)	1.57	1.23–2.00	0.0003
Allele mismatch in the GVH direction	1.57	1.01–2.43	0.043
OS			
Age ⩾ 50 years (vs age < 50 years)	1.45	1.27–1.66	< 0.0001
Use of *in vivo* TCD (vs no *in vivo* TCD)	0.50	0.35–0.73	0.0003
Performance status, 2–4 (vs 0–1)	2.36	1.99–2.79	< 0.0001
PBSC (vs BM)	1.41	1.23–1.61	< 0.0001
Disease risk, high (vs standard)	2.08	1.81–2.38	< 0.0001
Allele mismatch in the GVH direction	1.43	1.11–1.85	0.0058

Abbreviations: CI = confidence interval; HR = hazard ratio; NRM = non-relapse mortality; TCD = T-cell depletion.

mismatch: HR 2.12, 95% CI 1.36–3.30, $P < 0.0001$; multiple allele mismatches: HR 4.73, 95% CI 1.88–11.87, $P < 0.0001$). We also assessed the impact of each locus, excluding patients with multiple allele mismatches. The cumulative incidences of grade III–IV acute GVHD were 9.4% (95% CI 2.3–22.6%) in HLA-A mismatch, 38.9% (16.7–60.8%) in HLA-B mismatch and 19.7% (11.1–30.2%) in HLA-DRB1 mismatch (Figure 1f). In a multivariate analysis, the presence of HLA-B mismatch or HLA-DRB1 mismatch was associated with an increased risk of grade III–IV acute GVHD (HLA-A: HR 0.89, 95% CI 0.29–2.68, $P = 0.830$; HLA-B: HR 4.74, 95% CI 2.00–11.28, $P < 0.0001$; HLA-DRB1: HR 2.16, 95% CI 1.22–3.85, $P = 0.0009$).

To exclude the possibility that HLA antigen mismatch in the HVG direction may affect the incidence of acute GVHD, we performed a subgroup analysis that included patients without HLA antigen mismatch in the HVG direction. In this subgroup analysis, the cumulative incidences of grade II–IV and grade III–IV acute GVHD in the Mismatch group were significantly higher than those in the Match group (grade II–IV 41.3% vs 29.5%, $P = 0.010$; grade III–IV 24.0% vs 9.6%, $P < 0.0001$). In multivariate analyses, the presence of an HLA allele mismatch in the GVH direction was still associated with increased risks of grade II–IV and grade III–IV acute GVHD (HR 1.75, 95% CI 1.30–2.35, $P = 0.0002$; HR 2.39, 95% CI 1.60–3.58, $P < 0.0001$, respectively).

Graft failure

The cumulative incidence of neutrophil engraftment at 60 days was 96.3% (95% CI 95.4–97.0%) in the Match group and 90.4% (95% CI 83.6–94.5%) in the Mismatch group ($P = 0.0044$). Although the presence of HLA antigen mismatch in the HVG direction was associated with an increased risk of graft failure in a multivariate analysis (HR of engraftment 0.79, 95% CI 0.65–0.95, $P = 0.013$), the presence of at least one allele mismatch in the GVH direction was not associated with an increased risk of graft failure.

NRM and relapse

The cumulative incidences of NRM at 2 years were 13.7% (95% CI 12.3–15.3%) in the Match group and 19.2% (95% CI 12.8–26.6%) in the Mismatch group ($P = 0.022$, Figure 2a). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an increased risk of NRM (HR 1.64, 95% CI 1.11–2.41, $P = 0.012$, Table 2). The cohort with a one allele mismatch was associated with an increased risk of NRM, compared with the allele-matched cohort, in a multivariate analysis (one allele mismatch HR 1.83, 95% CI 1.18–2.84, $P = 0.0073$; multiple allele mismatch HR 0.93, 95% CI 0.22–3.94, $P = 0.92$). We also assessed the impact of each locus excluding patients with multiple allele mismatches. The cumulative incidences of 2-year NRM were 29.3% (95% CI 14.2–46.2%) in HLA-A mismatch, 23.5% (6.9–45.8%) in HLA-B mismatch and 15.1% (7.3–25.5%) in HLA-DRB1 mismatch (Figure 2b). In a multivariate analysis, the presence of an HLA-A mismatch was associated with an increased risk of NRM (HLA-A: HR 2.73, 95% CI 1.34–5.54, $P = 0.0056$; HLA-B: HR 2.08, 95% CI 0.74–5.88, $P = 0.17$; HLA-DRB1: HR 1.31, 95% CI 0.69–2.50, $P = 0.41$).

The cumulative incidences of relapse at 2 years were 32.7% (95% CI 30.7–34.7%) in the Match group and 30.1% (95% CI 22.3–38.3%) in the Mismatch group ($P = 0.54$, Figure 2c). The presence of allele mismatch did not affect the incidence of relapse. The cumulative incidences of relapse at 2 years were 22.9% (95% CI 9.7–39.3%) in HLA-A mismatch, 24.2% (6.9–47.0%) in HLA-B mismatch and 35.4% (23.6–47.4%) in HLA-DRB1 mismatch (Figure 2d). There was no statistically significant difference among the four groups.

OS

The probabilities of OS at 2 years after allogeneic HSCT were 61.7% in the Match group and 54.0% in the Mismatch group ($P = 0.0090$, Figure 2e). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an inferior OS (HR 1.43, 95% CI 1.11–1.85, $P = 0.0058$, Table 2). Other factors that were associated with an increased risk of overall mortality were age (⩾50 years), poor performance status (2–4), use of PBSC and high disease risk. Compared with an allele match, the presence of a one allele mismatch was associated with an inferior OS in a multivariate analysis (one allele mismatch: HR 1.46, 95% CI 1.11–1.90, $P = 0.0059$; multiple allele mismatch: HR 1.25, 95% CI 0.59–2.66, $P = 0.56$). We also assessed the impact of each locus excluding patients with multiple allele mismatches. The probabilities of 2-year OS were 57.6% (95% CI 38.0–72.9%) in HLA-A mismatch, 55.0% (29.8–74.5%) in HLA-B mismatch and 51.0% (37.7–62.9%) in HLA-DRB1 mismatch (Figure 2f). In a multivariate analysis, patients with an HLA-A or HLA-DRB1 mismatch tended to have a worse OS (HLA-A: HR 1.51, 95% CI 0.93–2.45, $P = 0.094$; HLA-B: HR 1.49, 95% CI 0.77–2.87, $P = 0.24$; HLA-DRB1: HR 1.43, 95% CI 1.00–2.03, $P = 0.050$).

DISCUSSION

In this study, we have demonstrated for the first time that HLA allele mismatch in the GVH direction in related HSCT was associated with increased risks of acute GVHD and NRM, which led to a poor OS. No previous study has assessed the impact of HLA allele mismatch in the related HSCT setting, as it is generally believed that HLA is completely matched in serologically HLA-matched related HSCT, especially in sibling donors if the parental HLA types are missing. Our result demonstrated that there is a possibility of HLA allele mismatch even in serologically matched related HSCT (5.6% in an HLA serologically matched donor/recipient combination). Our current result in related HSCT was consistent with the findings in unrelated HSCT, which suggests that serological HLA typing is insufficient to assess HLA

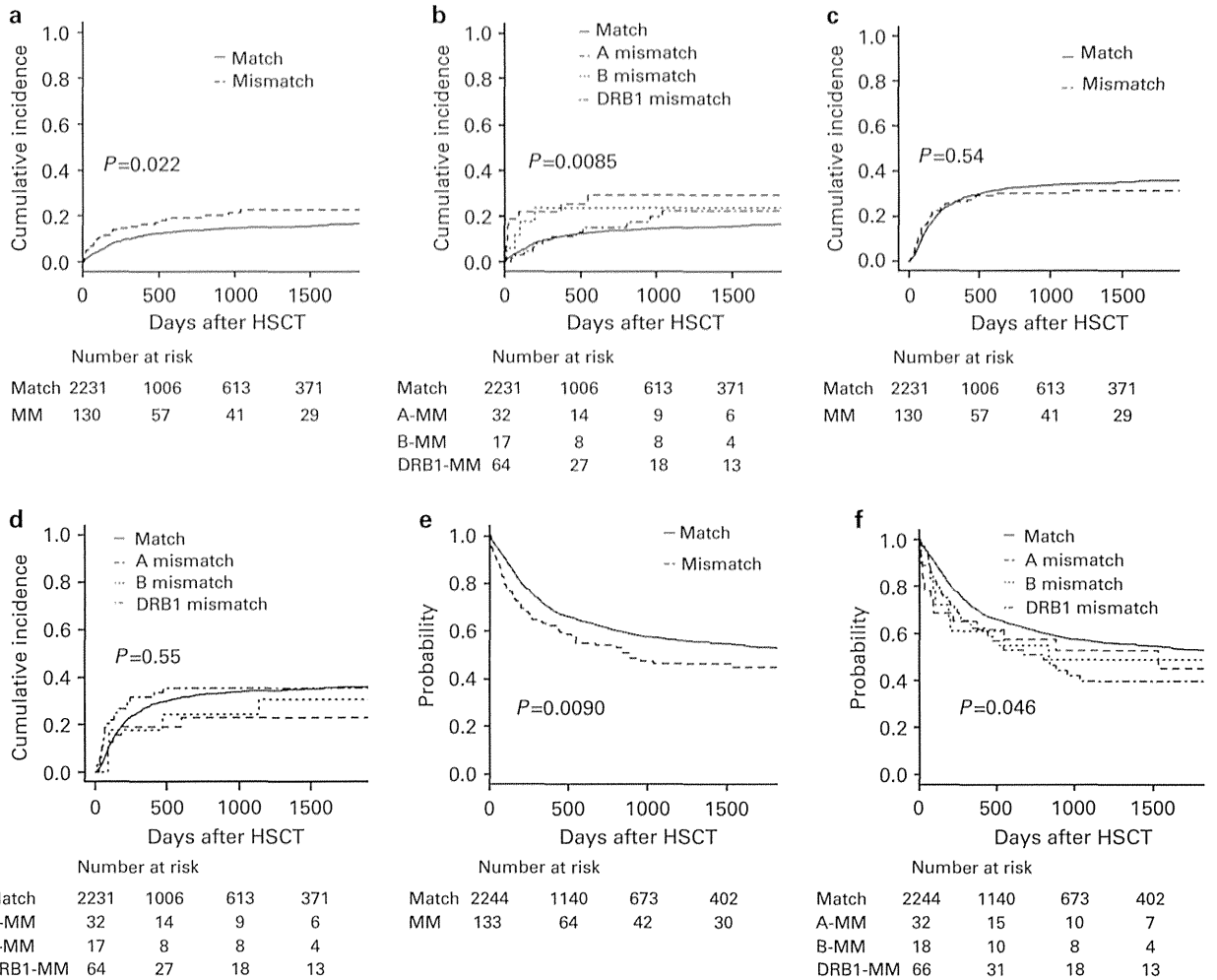

Figure 2. NRM, relapse and OS. Cumulative incidence of NRM grouped according to (**a**) allele mismatch and (**b**) locus of allele mismatch. Cumulative incidence of relapse grouped according to (**c**) allele mismatch and (**d**) locus of allele mismatch. The probability of OS grouped according to (**e**) allele mismatch and (**f**) locus of allele mismatches.

compatibility.[1,2] Therefore, HLA typing at high resolution (allele-level typing) should be done in all patients, including matched related transplants. The presence of HLA allele mismatch in the GVH direction should be taken into consideration when selecting a stem cell donor and determining the intensity of GVHD prophylaxis. In this study, the presence of HLA-B allele mismatch was associated with a significantly increased risk of severe acute GVHD. The significant impact of HLA-B antigen mismatch seemed to be similar to that in a previous report from Japan that assessed the impact of HLA-one antigen mismatch in related HSCT.[10] An important limitation here is the lack of HLA-C information in our current database. The frequency of an HLA-C mismatch in an HLA-B-mismatched group was shown to be substantially higher than those in the HLA-A and -DR antigen-mismatched groups.[15,16] In our database, information about the HLA-C allele was available in only 1152 cases (48.5%). Therefore, the impact of HLA-B and -C allele mismatch in related HSCT should be clarified in analyses using larger cohorts with complete HLA-C allele information.

One important issue in this study was the result that the use of PBSC was significantly associated with an increased risk of grade III–IV acute GVHD (HR 1.85, 95% CI 1.41–2.44, $P < 0.0001$, Table 2), which led to an increased risk of NRM and overall mortality. Therefore optimization of GVHD prophylaxis is particularly important in patients who receive PBSC to improve the clinical outcome.

A major limitation of this study is the small sample size in the Mismatch group, which is largely due to the fact that we included patients for whom data on the HLA allele were available. Because of the limited number of cases with HLA allele mismatch, it was difficult to assess the effect of the type of GVHD prophylaxis, such as the use of T-cell depletion, on the incidence of acute GVHD. Although the use of T-cell depletion seems to reduce the risk of GVHD, this association was not statistically significant (data not shown). This may have been due to the limited number of cases with T-cell depletion in this cohort.

In conclusion, our findings suggest that the presence of an HLA allele mismatch in serologically matched related HSCT was associated with increased risks of acute GVHD and NRM, which led to a poor OS. Therefore, HLA typing at high resolution (allele-level typing) should be done in all patients, including matched related transplants. The optimal GVHD prophylaxis in patients who receive stem cells from an HLA allele-mismatched related donor should be explored prospectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Park M, Seo JJ. Role of HLA in hematopoietic stem cell transplantation. *Bone Marrow Res* 2012; **2012**: 680841.
2 Petersdorf EW. Optimal HLA matching in hematopoietic cell transplantation. *Curr Opin Immunol* 2008; **20**: 588–593.
3 Morishima Y, Sasazuki T, Inoko H, Juji T, Akaza T, Yamamoto K *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**: 4200–4206.
4 Woolfrey A, Klein JP, Haagenson M, Spellman S, Petersdorf E, Oudshoorn M *et al.* HLA-C antigen mismatch is associated with worse outcome in unrelated donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2011; **17**: 885–892.
5 Kanda Y, Kanda J, Atsuta Y, Maeda Y, Ichinohe T, Ohashi K *et al.* Impact of a single human leucocyte antigen (HLA) allele mismatch on the outcome of unrelated bone marrow transplantation over two time periods. A retrospective analysis of 3003 patients from the HLA Working Group of the Japan Society for Blood and Marrow Transplantation. *Br J Haematol* 2013; **161**: 566–577.
6 Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M *et al.* High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 2007; **110**: 4576–4583.
7 Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M *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**: 1923–1930.
8 Ciurea SO, Saliba RM, Rondon G, Patah PA, Aung F, Cano P *et al.* Outcomes of patients with myeloid malignancies treated with allogeneic hematopoietic stem cell transplantation from matched unrelated donors compared with one human leukocyte antigen mismatched related donors using HLA typing at 10 loci. *Biol Blood Marrow Transplant* 2011; **17**: 923–929.
9 Valcarcel D, Sierra J, Wang T, Kan F, Gupta V, Hale GA *et al.* One-antigen mismatched related versus HLA-matched unrelated donor hematopoietic stem cell transplantation in adults with acute leukemia: Center for International Blood and Marrow Transplant Research results in the era of molecular HLA typing. *Biol Blood Marrow Transplant* 2011; **17**: 640–648.
10 Kanda J, Saji H, Fukuda T, Kobayashi T, Miyamura K, Eto T *et al.* Related transplantation with HLA-1 Ag mismatch in the GVH direction and HLA-8/8 allele-matched unrelated transplantation: a nationwide retrospective study. *Blood* 2012; **119**: 2409–2416.
11 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**: 269–274.
12 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
13 Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496–509.
14 Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452–458.
15 Prasad VK, Heller G, Kernan NA, O'Reilly RJ, Yang SY. The probability of HLA-C matching between patient and unrelated donor at the molecular level: estimations based on the linkage disequilibrium between DNA typed HLA-B and HLA-C alleles. *Transplantation* 1999; **68**: 1044–1050.
16 Petersdorf EW. The major histocompatibility complex: a model for understanding graft-versus-host disease. *Blood* 2013; **122**: 1863–1872.