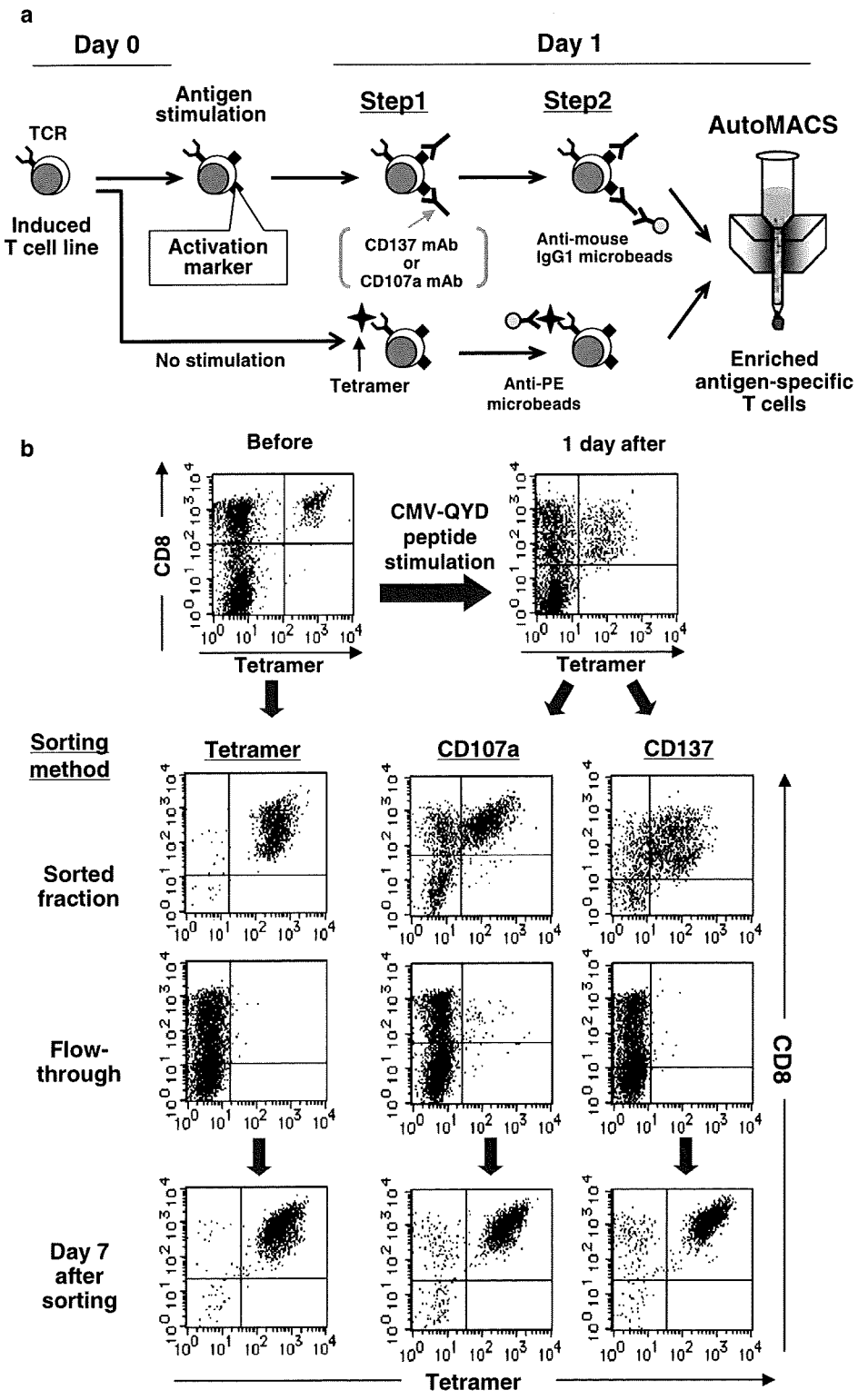

Fig. 3 Schematic illustration of positive selection using CD137, CD107a, or tetramer. **a** The MLPC-induced cell lines on days 14–16 after the initial stimulation were split and either restimulated with cognate antigenic peptide (2/3 part) or left without any stimulation (1/3 part) overnight. On the following day, T cells upregulating CD137 or CD107a by restimulation were first stained with individual antibodies and then incubated with anti-mouse IgG1 microbeads. T cells left untreated were first stained with cognate PE-conjugated tetramer and then incubated with anti-PE microbeads. T cells coated with the microbeads were then subjected to AutoMACS-based positive selection. **b** Representative flow cytometry data demonstrating the enrichment of CMV-QYD-specific T cells with the individual methods. The profiles of $CD8^+$ $tetramer^+$ cells in the AutoMACS-sorted, flow-through, and sorted fractions cultured for 7 days are shown

analyzed the phenotypes and functions of in vitro-expanded T cell lines obtained by the CD137 method. The CMV-QYD-specific T cell lines (gated by A24/CMV-QYD tetramer staining) were mostly $CD45RO^+$ and $CD45RA^-$, and more than a quarter of cells expressed both CCR7 and CD28, a hallmark for central memory

Table 1 Comparison of the recoveries of CMV/QYD-specific T cells among the three sorting methods

	% tetramer$^+$ cells (day 0)	Method	% CD137$^+$ or CD107a$^+$ among tetramer$^+$ cells	Number of tetramer$^+$ cells prior to sorting (day 1) ($\times 10^5$)[a]	Sorted fraction (day 1)		
					% tetramer$^+$	Number of tetramer$^+$ cells ($\times 10^5$)	% recovery of tetramer$^+$ cells
Experiment 1	8.3	CD137	95.6	6.4	66.3	3.7	58.0
		CD107a	95.0	6.4	58.4	3.3	51.1
		Tetramer	–	8.8	97.9	4.3	41.8
Experiment 2	12.4	CD137	98.3	1.74	80.8	1.29	74.1
		CD107a	87.3	1.74	75.2	0.98	56.3
		Tetramer	–	1.88	80.2	0.64	34.0
Experiment 3	22.5	CD137	99.2	23.8	96.0	2.88	12.1
		CD107a	11.3	23.8	32.1	0.29	1.2
		Tetramer	–	26.5	99.5	11.8	44.5

The experiment number corresponds to that shown in Fig. 4a

[a] Reduced number of tetramer$^+$ cells was caused mainly by activation-induced cell death during overnight stimulation with antigen

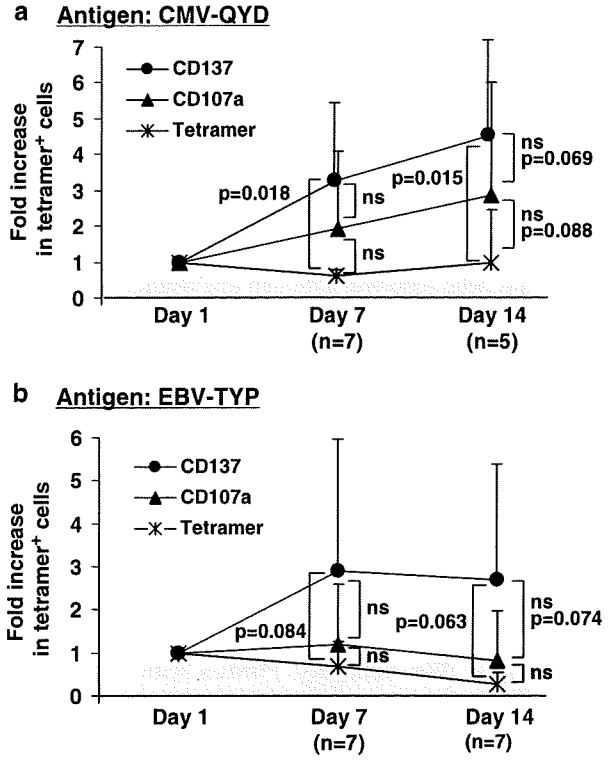

Fig. 4 Expansion of enriched cells after sorting with CD137, CD107a, or tetramers. AutoMACS-sorted fractions were cultured in a 24- or 96-well culture plate in ALyS505N-1000 media containing 1,000 U/ml IL-2 for the indicated period. Average fold increases of cognate tetramer$^+$ cells from seven individuals including three shown in Table 1 are shown. **a** Expansion of CMV-QYD-specific T cell lines. **b** Expansion of EBV-TYP-specific T cell lines. Statistical values were obtained using paired Student's *t* test. The *error bars* represent the mean SD of the seven experiments except one including five experiments for CMV-QYD on day 14. *ns* not significant

T cells (Fig. 5a). Upon stimulation with cognate peptide (CMV-QYD), nearly half of the T cells could produce IFN-γ (Fig. 5b). Finally, one of the T cell lines showed robust and specific lytic activity against CMV-QYD peptide-pulsed autologous B-LCLs (75% at an E/T ratio of 2, Fig. 5c).

3.6 Insufficient CD137 upregulation on antigen-stimulated CD4$^+$ T cells for positive selection

Since there is currently no feasible method to positively select antigen-specific CD4$^+$ cells, we examined whether CD137 might be sufficiently upregulated for MACS-based sorting. We first generated T cell lines by stimulating PBMC with an HLA-DRB1*0101-restricted EBV-TSL peptide. Figure 6a shows a representative kinetic profile of CD137 expression on a T cell line before and after restimulation with EBV-TSL peptide. Percentages of CD137$^+$ cells among (CD4$^+$) HLA-DRB1*0101/EBV-TSL tetramer$^+$ cells increased from 8.4 to 40.4% after 16 h of stimulation, and declined to 14.6% at 48 h. However, the (CD4$^+$) tetramer$^-$ fraction already showed upregulated CD137 expression before antigen stimulation, and its upregulation was more pronounced in terms of fluorescent intensity than that of the tetramer$^+$ fraction at 16 h, for unknown reasons (Fig. 6a, middle panel). As a result, although relatively more tetramer$^+$ CD137$^+$ cells were recovered in the sorted fraction (Fig. 6b, middle panels), the majority of tetramer$^+$ cells were eventually lost into the flow-through fraction, probably due to a weaker upregulation of CD137 insufficient for MACS-based sorting.

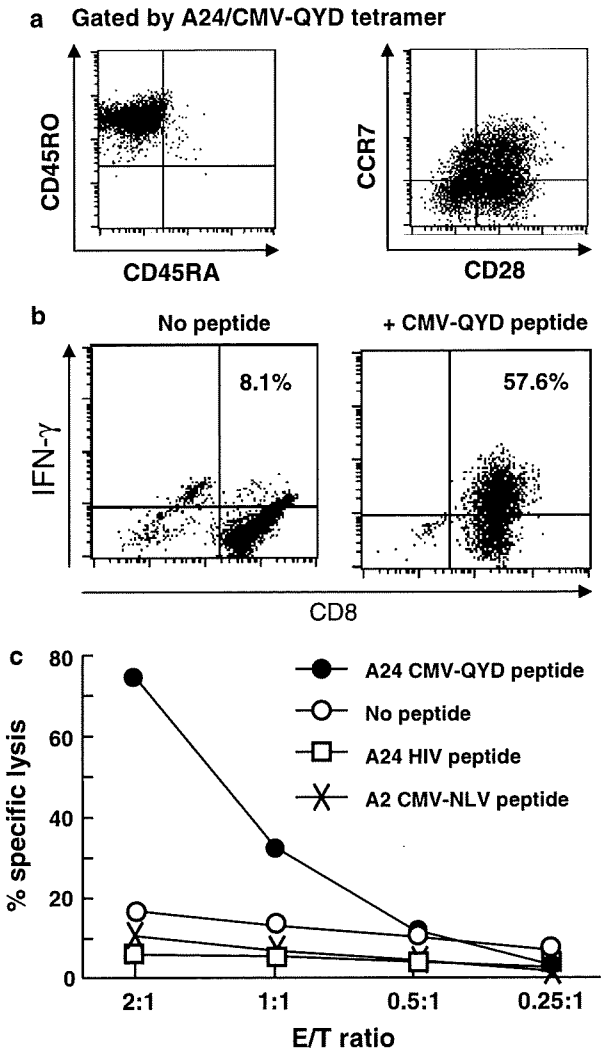

Fig. 5 Phenotypes and functions of CD137-sorted and 7-day cultured T cell lines. **a** Representative flow cytometry profile of CMV-QYD-specific T cell lines for differentiation markers. T cells gated for the cognate tetramer were analyzed with the indicated markers. **b** Capacity for IFN-γ production upon stimulation with autologous B-LCL pulsed with or without cognate peptide. **c** Cytotoxicity of T cell lines against peptide pulsed autologous B-LCL at the indicated effector:target (E:T) ratios. The data shown are representative of three independent experiments for **b** and **c**

4 Discussion

The enrichment of antigen-specific T cells is the first key step for successful adoptive immunotherapy, necessary to maximize efficacy and minimize unwanted reactivity to self-antigens that may result in autoimmunity. The present comparison of three methods (with CD137, CD107a, and HLA multimers) that can isolate T cells simply (i.e., by staining and separation with a MACS-based sorter) without any need for expensive flow cytometric cell sorters, showed a comparable recovery of antigen-specific $CD8^+$ cells assessed by cognate tetramer staining. However, the CD137-based method was superior when cell proliferation following enrichment was also taken into consideration (Fig. 4), although the difference between this and the CD107a-based method did not reach significance, possibly due to limited number ($n = 7$) of individuals tested and the inter-individual variation in the level of CD137 and CD107a upregulation after stimulation (data not shown). Nevertheless, the advantage of the CD137-based method is reasonable because CD137 has been shown to deliver a survival signal to activated T cells [14, 15]. In addition, CD137 was found to be upregulated in almost all (>90%) antigen-specific T cells, based on tetramer staining, when compared with CD107a (up to 70%), so that the former is likely to cover the full repertoire of antigen-specific T cells. Finally, we learned that CD137-based sorting is not suitable for antigen-specific $CD4^+$ T cells, at least with our current approach using simple "bulk" cultures, due to high background and bystander expression of CD137. However, CD137 was indeed upregulated upon antigen stimulation of cognate $CD4^+$ cells (Fig. 6a), as shown by others [16]. Because monocytes constitutively express CD137 (data not shown), the residual monocytes which were not killed by antigen-specific helper $CD4^+$ could contaminate the sorted fraction, likely resulting in the low-level purity of antigen-specific $CD4^+$ cells. To isolate antigen-specific $CD4^+$ helper T cells, the positive selection of CD154 or the CD40 ligand has been reported, although this method requires the addition of CD40-specific blocking antibodies to avoid the downregulation of CD154 induced by antigen stimulation [17]. We initially wished to isolate both antigen-specific $CD8^+$ and $CD4^+$ T cells with a single reagent, CD137, but our data demonstrated that it might be a suboptimal method at present, unless the IFN-γ secretion assay, which requires two more steps, is performed [11].

In the current study, to induce cell surface CD137 or CD107a expression with antigenic peptides, they were simply added directly to PBMC suspensions without antigen-presenting cells in order to minimize in vitro manipulation. We stimulated PBMCs with a commonly used concentration (i.e., 10 μg/ml) of antigenic peptides for simplicity because resting memory T cells in PBMCs are relatively resistant to AICD compared to activated effector T cells [18]. Restimulation of in vitro-activated T cells just before positive selection, however, did induce moderate reduction of cognate T cells (data not shown), possibly due to AICD [18] or T cell versus T cell killing [19], whereby antigen-specific T cells presenting the pulsed peptide are killed by other antigen-specific T cells. AICD could be avoided using more precisely titrated concentrations of peptides, but this might be difficult since the occurrence of AICD may also depend on other factors, including the T cell activation status, co-existing cytokines, and

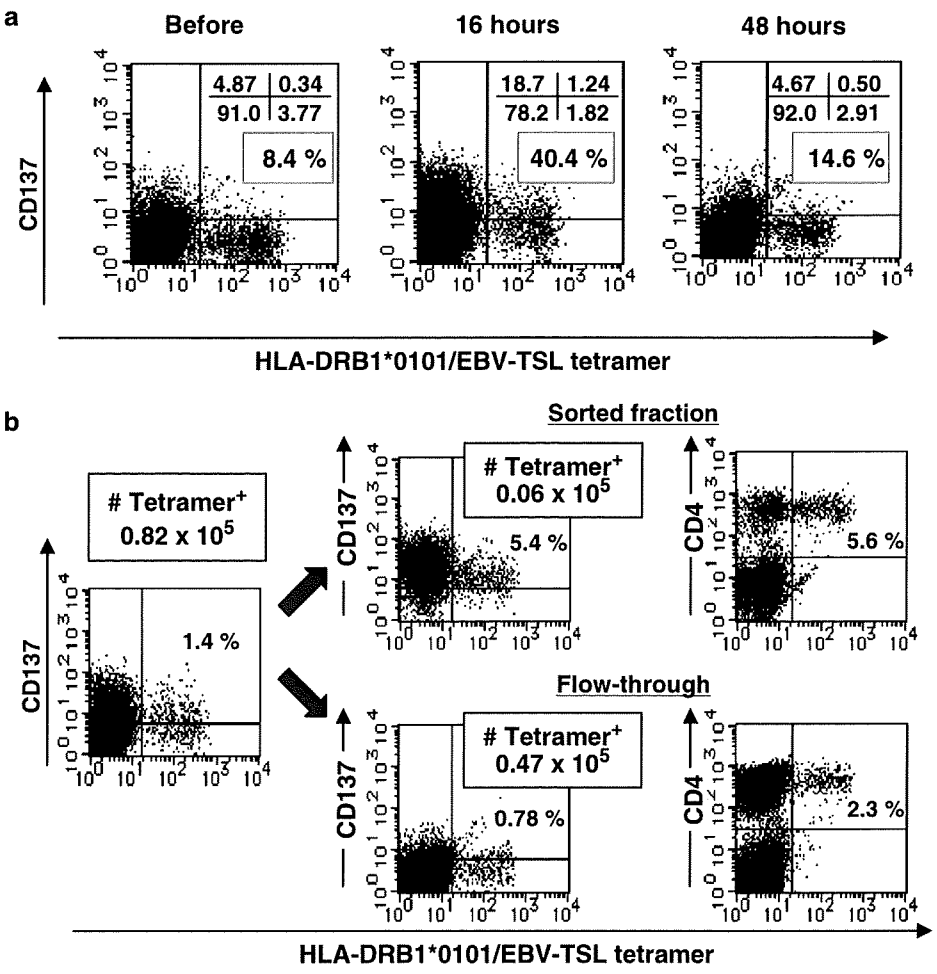

Fig. 6 Induction of CD137 expression on antigen-specific $CD4^+$ T-cells. **a** PBMCs were stimulated in MLPC with the HLA-DRB1*0101-restricted EBV-TSL peptide. On day 14 of culture, the T cells were stimulated with 10 ng/ml EBV-TSL peptide. The expression of CD137 was assessed along with HLA-DRB1*0101/EBV-TSL tetramer staining before and 16 and 48 h after stimulation. **b** Representative flow cytometry data demonstrating the enrichment of EBV-TSL-specific T cells with the CD137-based method. The profiles of $tetramer^+$ cells counterstained with either CD137 (*middle column*) or CD4 (*right column*) in the AutoMACS-sorted and flow-through fractions are shown. Numbers in *squares* represent the absolute numbers of $tetramer^+$ cells, indicating the loss of most antigen-specific T cells into the flow-through fraction

costimulatory molecules [13]. The latter "mutual" killing could be avoided using peptide-pulsed autologous antigen-presenting cells; however, any usage of cells, even autologous, requires multiple steps, including thawing, washing, peptide pulsing, and irradiation, with which the risk of bacterial contamination may increase. Thus, the optimization of simple and safe restimulation conditions for the maximal induction of CD137 or CD107a while minimizing the loss of antigen-specific T cells should be further explored.

As previously shown, CD137- and CD107a-based methods can be performed without prior knowledge of precise peptide sequences or HLA restriction, unlike the tetramer-based approach. Although we used predetermined CMV- and EBV-derived peptides as model antigens in this study, we also confirmed that T cell enrichment followed by the cloning of minor histocompatibility antigen-specific T cells are possible with CD107a- or CD137-based sorting after T cell lines are restimulated using endogenously antigen-expressing PBMCs or B-LCLs (our unpublished observations). This suggests that both methods are applicable for the positive selection of various T cell lines.

The long-term in vitro culture or expansion of T cells, especially after cloning, is known to be detrimental to T cell survival after returning to in vivo conditions due to progression to terminal differentiation [20]. Therefore, short-term induction culture, followed by enrichment and/or further short-term expansion are warranted. In our phenotypic and functional analyses, most T cells enriched with the CD137-based method and cultured for 7 days retained a central memory phenotype (Fig. 5a), IFN-γ production capacity, and cytolytic activity when challenged with cognate antigen-presenting cells (Fig. 5b, c). Thus, short-term culture for 7 days did not result in the loss of critical functions of T cells necessary for adoptive immunotherapy. It has been shown that an average ninefold expansion over 8 days is possible for CD137-enriched cells when cultured in the presence of IL-2, IL-7 and, IL-15 [12]. In our expansion study, only an average 2.6-fold expansion was obtained. The difference might be caused partly

because we did not use IL-7 and IL-15, especially the latter, which is known to deliver anti-apoptotic signals and augment the proliferation and homeostasis of memory $CD8^+$ T-cells [21]. The other reason could be that we sorted antigen-specific cells from memory T cell pools of CMV- or EBV-seropositive individuals while others have employed $CD45RA^+$ naïve cells as a source of antigen-specific T cells [12]. Collectively, our data demonstrate that CD137-based sorting is indeed superior to other "one step" sorting methods.

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LETTER TO THE EDITOR

Consulting clinic for related family donors in hematopoietic stem cell transplantation

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When patients with hematologic or other intractable disorders need to undergo allogeneic HSCT (hematopoietic stem cell transplantation), related family members are most often considered as candidates for the hematopoietic stem cell (HSC) donation. According to a nationwide questionnaire survey in Japan, 98.0% of family donors are informed about the HSC donation procedure only by the clinicians involved in the care of the HSC transplant recipient ('recipient clinicians'). Moreover, when written informed consent for HSC donation was obtained, the recipient clinicians and the recipients themselves were present with the prospective donors in 89.3 and 70.7% of cases, respectively.[1] We questioned whether in such situations the donors were truly agreeing to donate of their own free will, and whether their safety in terms of physical eligibility was considered sufficiently. There have been many studies on the safety and ethical issues facing unrelated volunteer HSC donors[2,3] and especially on issues regarding PBSC collection.[4–6] However, surprisingly, few studies so far have addressed these issues with special reference to related donors.[7] Therefore, we started a unique donor consultation program, 'Consulting Clinic for Related HSC Donors (CRD),' in which two clinicians ('donor clinicians') who are not directly involved in the care of the prospective recipients play a central role in securing the rights and safety of related family donors.

We herein introduce the CRD process at our hospital (Figure 1). At the CRD visit, the donor candidate watches a video program to obtain general information about HSCT and completes a questionnaire to elicit a medical history. Then the donor clinician comprehensively explains what HSC donation is, especially focusing on the risks associated with BM and PBSC harvesting in addition to their potential alternatives. After the written informed consent for the donation is obtained, the donor candidate undergoes unified medical examinations to evaluate eligibility for the planned HSC donation; thereafter, the donor clinician and another physician who is also uninvolved in the care of the prospective recipient meet together and discuss whether the examination results satisfy our institutional eligibility criteria. Decisions are classified into three groups: (A) 'qualified', (B) 'reserved' (requesting more consideration) and (C) 'unqualified'. The patient is informed of the decision by the recipient clinician, who reports that the donor candidate is 'qualified' or 'unqualified' without disclosing the reasons, while the donor candidate is

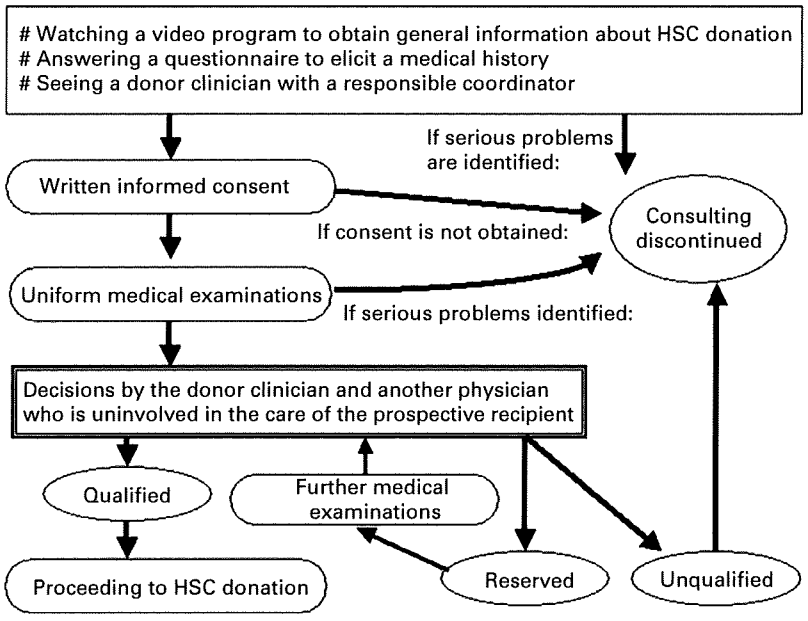

Figure 1 The diagram showing the process of our consulting clinic for related donors (CRD).

Table 1 Characteristics of donor candidates visiting our consulting clinic for related donors

Sex number (%)	
Male	38 (54)
Female	32 (46)
Median age, years (range, years)	48 (13–69)
Age group number (%)	
11–20 year	2 (3)
21–30 year	19 (27)
31–40 year	7 (10)
41–50 year	11 (16)
51–60 year	21 (30)
61–69 year	10 (14)
Relationship to the recipient number (%)	
Sibling	48 (69)
Son	8 (11)
Daughter	4 (6)
Father	3 (4)
Mother	7 (10)
Type of requested stem cell source number (%)	
Bone marrow	52 (74)
Peripheral blood	13 (19)
Bone marrow or peripheral blood	5 (7)

informed of the decision by the donor clinician. It is most important through this process for the donor clinician to avoid coercing the donation and to inform the donor candidates that they can cancel the donation at any time, even after providing written consent.

Between April 2003 and March 2007, a total of 70 donor candidates visited the CRD (Table 1). Two candidates were judged as 'unqualified' at the first medical interview. Sixty-eight qualification meetings by two clinicians were held to judge the eligibility of the candidates to undergo BM or PBSC donation; the initial decisions were 'qualified' in 43 cases (63%), 'reserved' in 21 (33%) and 'unqualified' in 4 (6%). The reasons for reservation were abnormalities in blood or urine examinations in 17 cases, suspicion of cardiovascular disease in 3 and suspicion of a neurological disorder in 1. The reasons for disqualification were suspicion of malignant tumors in three cases and presence of cardiovascular disease in one. Twenty of twenty-one candidates (95%) in the 'reserved' group were eventually judged 'qualified' after existing diseases were controlled and/or the donor was re-evaluated by another consultant physician or anesthesiologist. Three of four 'unqualified' candidates at the first CRD were reclassified as 'qualified' at the second CRD when the requested source of HSC was changed from PBSC to BM. Twelve of sixty-six 'finally qualified' donors (18%) did not actually donate HSCs due to various reasons, including death of the recipient before undergoing HSCT. To date, we have not observed any severe adverse events in the remaining 54 donors who actually donated HSC, with a median follow-up of 33 months (range, 5–51 months).

It is still difficult to estimate the contribution of our CRD to the improvement of donor safety because our experience is limited. However, we believe that our CRD has a meaningful impact on the ethical issues facing related donors. The psychological condition of the donor, particularly the donor's motives for considering the HSC donation, should be carefully assessed prior to giving informed consent. It is reported that family donor candidates may occasionally be subjected to coercion or external pressure, partly because such donors' desire to help relatives may be different from the motivations of unrelated donors.[8,9] In a survey of Japanese HSCT centers, 39.4% of related donors felt that they could not refuse to donate, while only 20% of them felt that they had a chance to refuse to donate HSCs if they did not want to do so.[1] Especially, in case the donor candidate is a child and the prospective recipient is one of their parents, there is a clear conflict of interest since the parent usually signs the consent for the child, raising a strong need for a guardian of the child donors.

In our CRD, several donor candidates hesitated over whether to agree or to refuse to donate. In those cases, the donor clinicians informed the candidate that even if the donation is refused, they would never tell the patient why the donor candidate could not donate HSC. This system largely put the candidates at their ease, and all of these candidates finally agreed to donate. Switzer *et al.*[10] reported that unrelated donors who felt they were pressured, irrespective of whether they were encouraged or discouraged to donate, are less likely to have a positive donation experience. In this way, our CRD program functioned as a buffer zone for the relationship between the donor and recipient.

In conclusion, we believe that this type of CRD program, which supports and respects the donors' free will, should become more widely used to secure the rights and safety of related donors. Further studies on the role of the CRD in the management of related familial donors are warranted.

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H Muramatsu[1], S Kimura[2], T Ichinohe[1], E Ashihara[2], T Ishikawa[1], T Maekawa[2] and T Uchiyama[1]
[1]*Department of Hematology and Oncology, Kyoto University Hospital, Kyoto, Japan and*
[2]*Department of Transfusion Medicine and Cell Therapy, Kyoto University Hospital, Kyoto, Japan*
E-mail: nohe@kuhp.kyoto-u.ac.jp

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Impact of ABO mismatching on the outcomes of allogeneic related and unrelated blood and marrow stem cell transplantations for hematologic malignancies: IPD-based meta-analysis of cohort studies

Junya Kanda, Tatsuo Ichinohe, Keitaro Matsuo, Richard J. Benjamin, Thomas R. Klumpp, Primoz Rozman, Neil Blumberg, Jayesh Mehta, Sang-Kyun Sohn, and Takashi Uchiyama

BACKGROUND: The impact of donor-recipient ABO matching on outcomes after allogeneic stem cell transplantation has been a matter of controversy.
STUDY DESIGN AND METHODS: Individual patient data–based meta-analysis was conducted with a pooled data set provided through six published and one unpublished cohorts. Outcomes in recipients of peripheral blood or bone marrow transplantation for hematologic malignancies were evaluated. A multivariate Cox model was used to adjust differences in outcomes of patients receiving ABO-matched grafts with those receiving major, minor, or bidirectional mismatched grafts. Considering multiple testing, p values of less than 0.05 and 0.001 were considered significant for the primary and secondary endpoints, respectively.
RESULTS: In all, 1208 cases, including 697 ABO-matched and 202 major, 228 minor, and 81 bidirectional mismatched transplants, were analyzed. Overall, adverse impact of ABO matching on overall survival (OS), as a primary endpoint, was not observed (adjusted hazard ratios [95% confidence intervals]: major, 1.03 [0.82-1.30], p = 0.81; minor, 1.19 [0.97-1.47], p = 0.10; bidirectional, 1.25 [0.91-1.72], p = 0.17). Among related stem cell recipients, ABO matching had no significant influence on OS, while the minor and bidirectional mismatched groups among unrelated stem cell recipients exhibited lower OS with marginal significance, especially in patients with acute leukemia, patients who received transplants after 1998, and patients who underwent transplants at Asian centers.
CONCLUSIONS: Our meta-analysis demonstrates no adverse association between any ABO mismatching and survival. However, marginally lower OS found in recipients of minor or bidirectional mismatched grafts from unrelated donors suggested the need for larger studies focusing on unrelated transplants.

ABO matching between donor and recipient in solid organ transplantation is generally thought to be essential for better outcomes.[1] In contrast, blood or marrow stem cell transplantation (SCT) from an ABO-mismatched donor is sufficiently

ABBREVIATIONS: AL = acute biphenotypic or unclassifiable leukemia; ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CLL = chronic lymphocytic leukemia; CML = chronic myelogenous leukemia; HR(s) = hazard ratio(s); IPD = individual patient data; MDS = myelodysplastic syndrome; ML = malignant lymphoma; MM = multiple myeloma; OS = overall survival; SCT = stem cell transplantation; TRM = treatment-related mortality.

From the Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; the Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; the Department of Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan; Biomedical Services, National Headquarters, American Red Cross, Washington, DC; the Department of Medicine, Temple University School of Medicine, Philadelphia, Pennsylvania; the Department of Immunohaematology, Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia; the Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, New York; The Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois; and the Department of Hematology and Oncology, Kyungpook National University Hospital, Daegu, Republic of Korea.

Address reprint requests to: Junya Kanda, MD, Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University; 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan; e-mail: jkanda16@kuhp.kyoto-u.ac.jp.

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feasible and is performed in routine clinical practice. However, several complications have been reported in ABO-mismatched SCT. Major mismatched transplantation, characterized by the presence of preformed anti-donor hemagglutinin, is sometimes complicated by delayed red blood cell (RBC) engraftment and pure red cell aplasia[2-7] and by hemolytic anemia.[8,9] In minor mismatched transplantation, characterized by the ability of donor B lymphocytes to produce anti-recipient hemagglutinin, acute hemolytic anemia, known as passenger lymphocyte syndrome, can occur shortly after SCT.[9-12] In bidirectional mismatched transplantation, characterized by the combination of major and minor characteristics, both sets of complications can occur. Owing to these reasons, clinicians are very interested in determining whether ABO mismatching affects the final outcome of SCT, especially when several donor candidates with various ABO-matching pairs are available. To resolve these issues, the impact of ABO mismatching on overall survival (OS) in SCT settings has been evaluated in many studies; however, all these studies obtained conflicting results. Some studies reported the association of poorer OS,[13-16] increased nonrelapse mortality,[17] or increased incidence of acute graft-versus-host disease (GVHD) with a single or any type of ABO mismatch compared with ABO-matched SCT.[16,18] In contrast, one report indicated better OS and decreased relapse rate in ABO-mismatched transplantation.[19] In addition to these contradictory reports, many studies reported that ABO mismatching had no impact on OS, incidence of acute GVHD, or relapse rate in SCT.[2,20-26] These contradictory results could have originated due to the following reasons: 1) in many studies, each ABO-mismatched pair is not analyzed independently; 2) the number of bidirectional mismatched transplants is often small; 3) transplant centers may employ differing treatment and supportive care regimes; and 4) the background of the studied populations is heterogeneous. To obtain more robust results, a few large retrospective studies analyzing more than 1000 patients have recently been performed. Seebach and coworkers[18] showed no impact of ABO mismatching on OS in an analysis of 3103 patients who had received bone marrow transplantation from a human leukocyte antigen (HLA)-identical sibling for early-stage acute leukemia and chronic myelogenous leukemia (CML). On the other hand, Michallet and colleagues[27] demonstrated an adverse impact of a minor mismatch on OS by analyzing 1108 patients who received SCT with a reduced-intensity conditioning regimen. Therefore, these results need further evaluation with other methods or populations. To reevaluate and summarize conflicting results from previously published studies and to provide better evidence, we designed a meta-analysis based on individual patient data (IPD) with a pooled data set. IPD-based meta-analysis is a relatively new approach to systemic reviews, aimed to reduce the bias in systemic reviews compared to meta-analysis based on abstracted data without IPD retrieval during central collection and reanalysis of IPD from each study.[28,29] We conducted the IPD-based meta-analysis using data sets, including those obtained from six previously published articles as well as an unpublished data set from one center that did not participate in previous studies.

MATERIALS AND METHODS

Study design

An IPD-based meta-analysis was designed to evaluate the impact of donor-recipient ABO matching on clinical outcomes after peripheral blood and marrow SCT for hematologic malignancies. The primary endpoint was OS, which was compared among patients receiving an ABO-matched graft and those receiving a major, minor, or bidirectional ABO-mismatched graft. The other endpoints analyzed were treatment-related mortality (TRM); GVHD-related mortality; and engraftment of reticulocytes, neutrophils, and platelets (PLTs).

Selection of studies for meta-analysis

Inclusion criteria for the selection of studies were as follows: 1) the studies were original articles published in English after 1995 and 2) the endpoints considered by the studies included the comparison of OS between ABO-matched and any mismatched SCTs. Exclusion criteria were as follows: 1) the studies included 80 or fewer SCT subjects and 2) the median follow-up period of the studies was less than 6 months. An initial literature search of the PubMed database was conducted using the following free-text terms: ABO blood-group system* and ("blood grouping and crossmatching"[Mesh] or blood group incompatibility*[Mesh]) and (bone marrow transplantation*[Mesh] or hematopoietic stem cell transplantation*[Mesh] or peripheral blood stem cell transplantation*[Mesh]). The date of the last search was June 30, 2007. The initial PubMed literature search identified 194 articles published between 1970 and 2007; 11 articles were found to be eligible for the analysis (Fig. 1).[13-16,18-24] Letters were sent to the corresponding authors of these 11 articles asking them to join the IPD-based meta-analysis and 6 of the corresponding authors agreed to participate. The 6 participating studies included 2 multicenter studies,[13,14,20,22-24] and the other 5 nonparticipating studies included 3 multicenter retrospective studies.[15,16,18,19,21] Patients receiving SCT from unrelated donors were present in 4 of the 6 participating studies and in 4 of the 5 nonparticipating studies. Two of the nonparticipating studies were relatively large, analyzing data of more than 1000 patients. In addition, Kyoto University, where this study was designed, participated in the study,

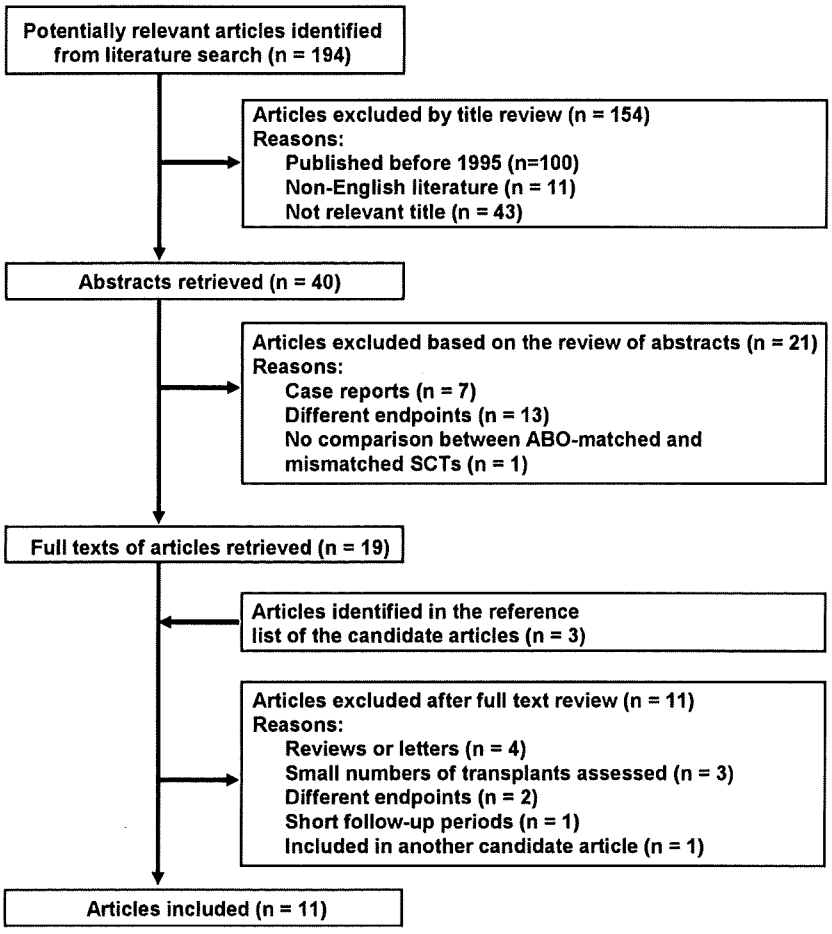

Fig. 1. A flow chart illustrating the process of article selection.

providing its data set on SCT that had not been subjected to survival analysis with reference to ABO matching.

Data collection

We first established the following exclusion criteria for IPD collection: 1) patients who did not meet the minimum data requirements in the following criteria, 2) patients who received SCT for diseases other than hematologic malignancies, 3) patients who received cord blood graft or both peripheral blood and marrow graft, and 4) patients who had experienced prior SCT or had no information regarding their SCT history. Further, we also excluded patients enrolled in the other pooled cohort studies so that the results of our study can be interpreted independently. Second, we defined all the variables required in the present study and made a report form for this data. We then asked the corresponding authors of the participating studies to fill the forms with data. Some authors sent all the raw data sets, which were converted to the report format of our study at the center. Ambiguous definitions were discussed and resolved with the principal investigators, corresponding authors, or data managers. Data from each study were verified against the reported results in some centers, and queries were resolved with the principal investigator, corresponding authors, data managers, or statisticians. The minimum data requirements for participation in this study were data on age and sex of recipients, diagnosis (acute myelogenous leukemia [AML], acute lymphoblastic leukemia [ALL], acute biphenotypic or unclassifiable leukemia [AL], CML, chronic lymphocytic leukemia [CLL], myelodysplastic syndrome [MDS], malignant lymphoma [ML], or multiple myeloma [MM]), type of stem cell source (marrow or peripheral blood stem cell), type of donor (related or unrelated), status of survival (alive, dead, or censored), days of survival after transplantation at the latest follow-up period, and donor-recipient ABO matching (matched or major, minor, or bidirectional mismatched pairs). Additional information requested included donor-recipient compatibility of HLA-A, HLA-B, and HLA-DR antigens by low-resolution typing (matched or mismatched); intensity of conditioning regimen (reduced intensity or myeloablative intensity); GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other prophylaxes); primary cause of death (disease progression or treatment-related death or detailed information regarding primary cause of death); disease status at SCT; and days to reticulocyte, neutrophil, and PLT engraftment. Data were excluded for patients who met any of the following criteria: patients undergoing SCT for other than hematologic malignancies, those receiving cord blood transplant, those with a history of prior SCT, or those included in a previous large multicenter study published before June 30, 2007. This study was approved by the institutional review board of Kyoto University and other institutions.

Definition of disease risks, engraftment, and primary cause of death

Complete remission in AML, ALL, AL, CLL, ML, and MM; chronic phase in CML; and untreated or complete remission in MDS were considered indicative of standard-risk diseases. Statuses other than complete remission in AML, ALL, AL, CLL, ML, and MM; accelerated phase and blastic crisis in CML; and statuses other than complete remission

in MDS after treatment were considered indicative of high-risk diseases. As described in previous studies,[2,5] the day of reticulocyte engraftment was defined as the first day when the percentage of reticulocytes in peripheral blood exceeded 1 percent. The day of neutrophil engraftment was defined as the first day of 3 consecutive days when the absolute neutrophil count exceeded 0.5×10^9 per L and that of PLT engraftment, the first day of 3 consecutive days when the PLT count exceeded 20×10^9 per L without PLT transfusions. The primary cause of death was classified into two categories: disease-associated death or treatment-related death. Among patients who experienced treatment-related death, GVHD-related death was defined as death primarily associated with acute or chronic GVHD.

Statistical analysis

Patient and transplant characteristics among ABO matching groups were compared by using Kruskal-Wallis test or chi-square analysis, as appropriate. Survival was estimated according to Kaplan-Meier product limit methods. Cumulative incidences of TRM, GVHD-related mortality, and engraftment were assessed using methods described elsewhere to eliminate the effect of competing risk.[30] The competing event in cumulative incidence analyses was defined as death without an event of interest. Disease-associated death was considered a competing risk in the analysis of cumulative incidence of TRM. Death other than GVHD-related death was considered a competing risk in the analysis of cumulative incidence of GVHD-related death. When appropriate, Gray's test was applied to assess the impact of the factor of interest. Multivariate proportional hazard modeling of subdistribution functions in competing risks was applied to assess the impact of potential prognostic factors.[31] Cox regression analysis was used to determine the impact of ABO matching on the primary endpoint with adjustment for age (continuous), sex (male or female), and center effects in the seven data sets. When appropriate, the following items were added as confounders in addition to age, sex, and center effects: diagnosis (acute leukemia or others), risk (standard-risk, high-risk, or unknown), donor (related or unrelated), stem cell source (bone marrow or peripheral blood), conditioning regimen (reduced intensity, myeloablative intensity, or unknown), GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or unknown), transplant year (1990-1997, 1998-2007, or unknown), and transplant centers (Asian or non-Asian centers). All of the confounders were also considered in the multivariate analysis of TRM, GVHD-related mortality, and engraftment. p Values of less than 0.05 were considered significant for the comparison of baseline characteristics and the primary endpoint. With regard to secondary endpoints, p values of less than 0.001 were considered significant to eliminate false-positive associations possibly induced by multiple testing, and p values of less than 0.05 and equal to 0.001 or more were defined as marginally significant. All analyses were conducted using computer software (STATA, Version 10, STATA Corp., College Station, TX; R, Version 2.6.3, The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Collection of data

Seven data sets containing data on a total of 1424 SCT patients were collected from six published data sets and one unpublished data set from one center. A total of 133 patients not meeting the minimum data requirements or those who received SCT for diseases other than hematologic malignancies were excluded. Twenty-eight patients who received cord blood graft or both peripheral blood and marrow graft were also excluded. In addition, 6 patients enrolled in the other pooled studies were excluded. Forty-nine patients who had experienced prior SCT or had no information regarding their SCT history were also excluded. In the end, 1208 transplants, including 697 ABO-matched cases and 202 major, 228 minor, and 81 bidirectional mismatched cases, were included in the study. With regard to the additional data requests, data on disease status at transplant were obtained for five data sets; type of conditioning regimen, GVHD prophylaxis, and transplant year for six data sets; reticulocyte engraftment for two data sets; neutrophil and PLT engraftment for five data sets; and binary data on either disease-associated death or treatment-related death for one data set and for five data sets with detailed information on the primary cause of death.

Characteristics of patients and transplants

Table 1 shows the patient characteristics. The cases included 709 related SCTs and 184 unrelated SCTs from Western centers as well as 214 related SCTs and 101 unrelated SCTs from Asian centers. The median age of the recipients was 39 years (range, 1-69 years). Marrow and peripheral blood stem cell was used for 915 and 293 cases, respectively. There were no significant differences among ABO-matched and mismatched groups for any category except for the type of donors and centers of transplantation. With regard to donor type, bidirectional ABO-mismatched grafts were more frequently used among unrelated SCTs when compared to the ABO-matched group. With regard to transplant centers, SCTs from bidirectional mismatched donors were more frequently performed in Asian centers.

OS

The median follow-up period of survivors was 37 months (range, 3-268 months). The unadjusted probabilities of OS

TABLE 1. Characteristics of patients and transplants

Characteristic	Match (%) (n = 697)	Major mismatch (%) (n = 202)	Minor mismatch (%) (n = 228)	Bidirectional mismatch (%) (n = 81)	p Value
Age					
Median (range)	39 (1-67)	39 (1-66)	39 (2-69)	43 (4-62)	0.074
Sex					
Male	393 (56.4)	129 (63.9)	118 (51.8)	45 (55.6)	0.087
Female	304 (43.6)	73 (36.1)	110 (48.3)	36 (44.4)	
Diagnosis					
AML/MDS	323 (46.3)	70 (34.7)	102 (44.7)	37 (45.7)	0.115
ALL	102 (14.6)	36 (17.8)	45 (19.7)	14 (17.3)	
AL	6 (0.9)	1 (0.5)	0 (0.0)	0 (0.0)	
CML	168 (24.1)	58 (28.4)	50 (21.4)	17 (21.0)	
CLL	5 (0.7)	6 (3.0)	4 (1.8)	0 (0.0)	
ML	67 (9.6)	26 (12.9)	18 (7.9)	10 (12.4)	
MM	26 (3.7)	5 (2.5)	9 (4.0)	3 (3.7)	
Risk					
Standard	341 (48.9)	75 (37.1)	91 (39.9)	39 (48.2)	0.597
High	112 (16.1)	31 (15.4)	50 (21.9)	17 (21.0)	
Unknown	244 (35.0)	96 (47.5)	87 (38.2)	25 (30.9)	
Type of donors					
Related					<0.001
HLA-matched	374 (53.7)	83 (41.1)	103 (45.2)	31 (38.3)	
HLA-mismatched	31 (4.5)	8 (4.0)	9 (4.0)	5 (3.7)	
HLA matching unknown	168 (24.1)	49 (24.3)	51 (22.4)	11 (13.6)	
Unrelated					
HLA-matched	121 (17.4)	62 (30.7)	63 (27.6)	31 (38.3)	
HLA-mismatched	3 (0.4)	0 (0.0)	2 (0.9)	3 (3.7)	
Stem cell source					
BM	519 (74.5)	155 (76.7)	177 (77.6)	64 (79.0)	0.649
PB	178 (25.5)	47 (23.3)	51 (22.4)	17 (21.0)	
Conditioning regimens					
Reduced intensity	101 (14.5)	27 (13.4)	41 (18.0)	8 (9.9)	0.209
Myeloablative intensity	515 (73.9)	144 (71.3)	158 (69.3)	69 (85.2)	
Unknown	81 (11.6)	31 (15.4)	29 (12.7)	4 (4.9)	
GVHD prophylaxis regimen					
CyA based	413 (59.3)	120 (59.4)	122 (53.6)	44 (56.8)	0.052
FK based	153 (22.0)	44 (21.8)	69 (30.3)	29 (35.8)	
Others	3 (0.4)	0 (0.0)	0 (0.0)	1 (1.2)	
Unknown	128 (18.4)	38 (18.9)	37 (16.2)	5 (6.2)	
Transplant year					
1990-1994	123 (17.7)	32 (15.8)	30 (13.2)	8 (9.9)	0.065
1995-1997	189 (27.1)	74 (36.6)	74 (32.5)	25 (30.9)	
1998-2000	147 (21.1)	40 (19.8)	36 (15.8)	18 (22.2)	
2001-2003	102 (14.6)	30 (14.9)	36 (15.8)	15 (18.5)	
2004-2007	58 (8.3)	15 (7.4)	31 (13.6)	12 (14.8)	
Unknown	78 (11.2)	11 (5.5)	21 (9.2)	3 (3.7)	
Transplant centers					
Asian centers	169 (24.3)	46 (22.8)	67 (29.4)	33 (40.7)	0.007
Non-Asian centers	528 (75.8)	156 (77.2)	161 (70.6)	48 (59.3)	

BM = bone marrow; CyA = cyclosporine; FK = tacrolimus; PB = peripheral blood.

(95% confidence interval [CI]) at 5 years among patients receiving ABO-matched grafts and major, minor, and bidirectional mismatched grafts were 48% (44%-52%), 48% (40%-56%), 45% (38%-51%), and 37% (26%-49%), respectively (Fig. 2A). Because different backgrounds and heterogeneity of results in stem cell sources were found, the impact of ABO matching among recipients of either related or unrelated SCT in each stratified category was assessed (Figs. 2B and 2C and 3A and 3B).

Among recipients of related SCT, no significant difference in OS was observed between the ABO-matched group and any other mismatched group. These results were consistent across each stratified group. In contrast, minor and bidirectional mismatched groups among unrelated SCT recipients tended to be associated with poorer OS when adjusted for age and sex (adjusted hazard ratio [HR]: minor, 1.71 [95% CI, 1.15-2.53], p = 0.008; bidirectional, 1.73 [95% CI, 1.05-2.86], p = 0.031). The adverse impact of minor and bidirectional mismatched grafts on OS in unrelated SCT was strongly observed in the following stratified categories: patients with acute leukemia, patients who received SCT after 1998, and patients who underwent transplants at Asian centers.

In multivariate regression analysis of OS adjusted for potential confounders listed in Table 2, no adverse impact of ABO matching on OS was observed among all or the

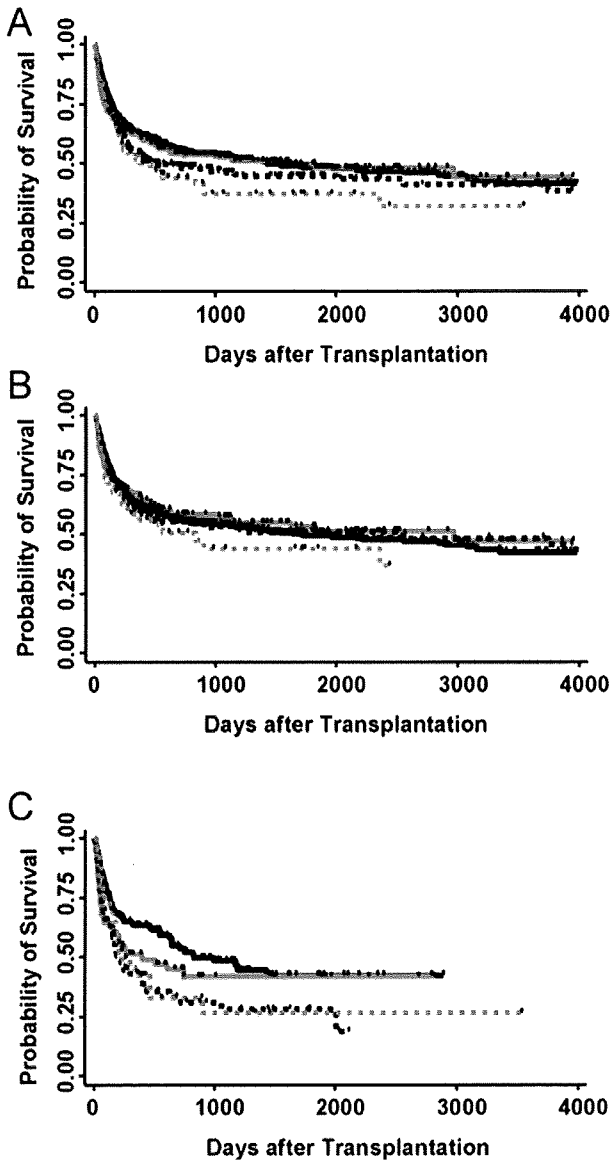

Fig. 2. Kaplan-Meier survival estimates of OS in all patients (A), those who received a related graft (B), and those who received an unrelated graft (C). (—) ABO-matched transplantation; (−) major mismatched; (· · ·) minor mismatched; (-··-) bidirectional mismatched.

subset of related SCTs, while minor and bidirectional mismatched groups showed tendency of poorer OS among the subset of unrelated SCT (adjusted HR: major, 1.38 [95% CI, 0.87-2.17], p = 0.17, minor, 1.68 [95% CI, 1.12-2.51], p = 0.012; bidirectional, 1.81 [95% CI, 1.08-3.00], p = 0.023) (Table 2).

TRM

Data on the primary cause of death were available for 1026 patients (85%). To evaluate the effect of ABO mismatch on treatment-related complications, we analyzed overall TRM at 5 years and early TRM within 100 days of transplantation. Although the cumulative incidences of overall TRM among the ABO-matched group and any mismatched groups did not show any significant difference in multivariate regression analysis, an increased risk of early TRM was observed among the bidirectional mismatched group (adjusted HR: 2.08 [95% CI, 1.14-3.79], p = 0.017; Table 3). This impact remained marginally significant among recipients of related SCTs (adjusted HR: 2.08 [95% CI, 1.04-4.15], p = 0.038). To evaluate whether early TRM was associated with acute GVHD, GVHD-related mortality within 100 days was analyzed using the available data sets (964 patients, 80%). Based on multivariate regression analysis adjusted for the confounding factors, the risk of acute GVHD-related mortality was significantly higher for the bidirectional mismatched group (adjusted HR, 9.35 [95% CI, 3.24-26.93], p < 0.001); however, further stratification by donor type could not be performed due to insufficient number of the data sets.

TABLE 2. Impact of ABO mismatching on OS

	OS (n = 1208)	
Category	HRs (95% CI)*	p Value
Overall		
Match	1.00	
Major	1.03 (0.82-1.30)	0.81
Minor	1.19 (0.97-1.47)	0.10
Bidirectional	1.25 (0.91-1.72)	0.17
Related SCT		
Match	1.00	
Major	0.93 (0.70-1.23)	0.62
Minor	1.02 (0.79-1.32)	0.88
Bidirectional	1.09 (0.71-1.68)	0.70
Unrelated SCT		
Match	1.00	
Major	1.38 (0.87-2.17)	0.17
Minor	1.68 (1.12-2.51)	0.012
Bidirectional	1.81 (1.08-3.00)	0.023

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

Engraftment

The data on days to reticulocyte, neutrophil, and PLT engraftment were available for 269 (24%), 667 (55%), and 662 (55%) patients, respectively. As shown in Table 4, multivariate regression analysis adjusted for confounders revealed no impact of ABO mismatching on reticulocyte, neutrophil, or PLT engraftment among patients who received related SCTs. In contrast, there was a marginally significant impact of ABO matching among recipients of unrelated SCTs. This analysis demonstrated a marginally significant impact of minor and bidirectional mismatched grafts on delay in reticulocyte engraftment compared to matched grafts among unrelated SCT recipients (major,

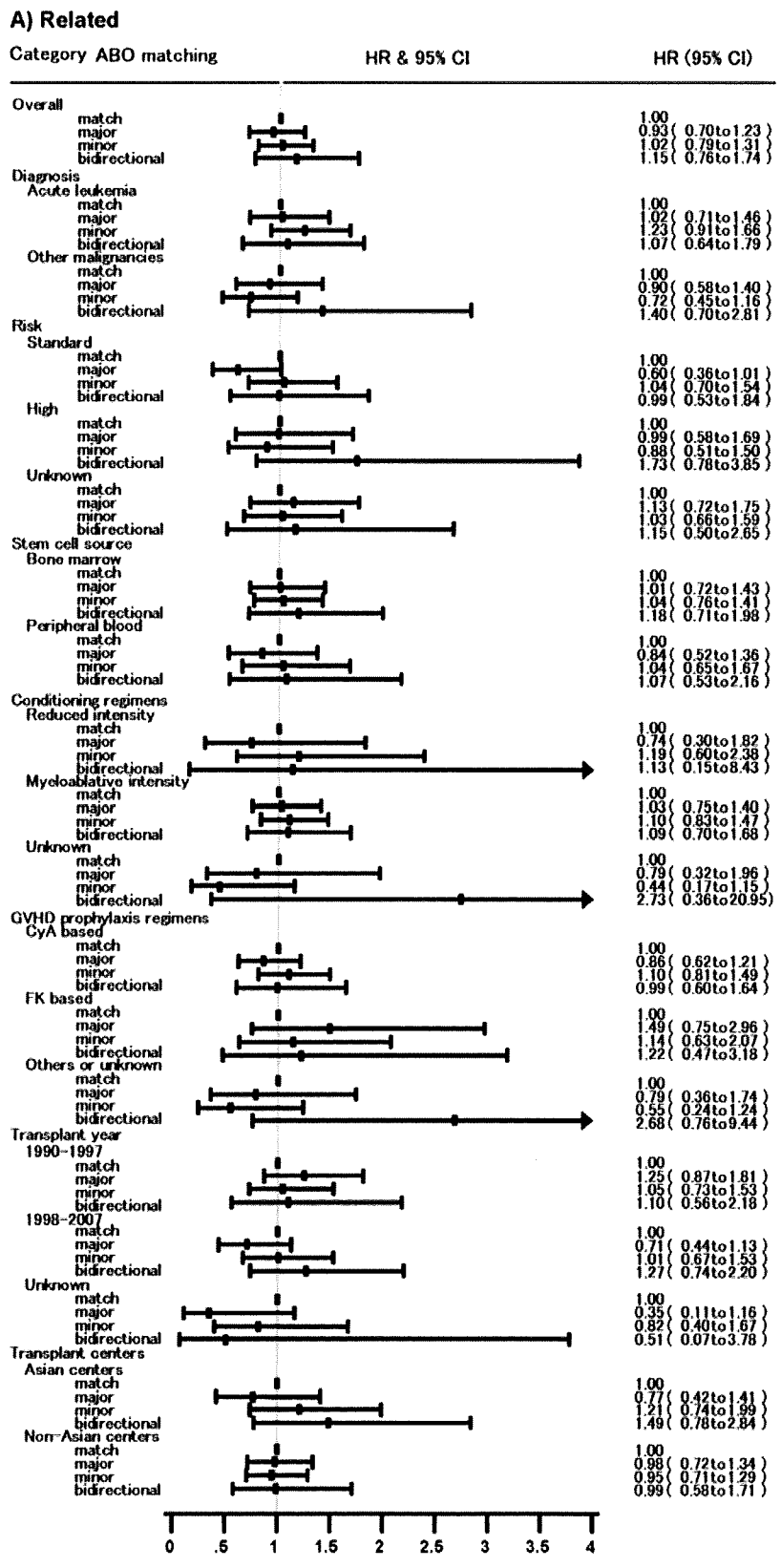

Fig. 3. Impact of ABO mismatching on OS in each stratified category among related (n = 923) (A) and unrelated stem cell transplantation (n = 285) (B). HRs were adjusted for age and sex. Square boxes on lines indicate HRs, and horizontal lines represent 95% CI.

p = 0.010; bidirectional, p = 0.012). Among recipients of unrelated SCTs, neutrophil engraftment tended to be delayed in the bidirectional mismatched group compared to the matched group (p = 0.019), and PLT engraftment tended to be delayed in the minor and bidirectional mismatched groups when compared to the matched group (minor mismatch, p = 0.023; bidirectional, p = 0.024).

DISCUSSION

To integrate the previous contradictory results, and to provide new data regarding the impact of ABO matching on survival after allogeneic blood and marrow SCTs, we performed an IPD-based meta-analysis using seven independent data sets including more than 1200 ABO-matched and mismatched transplants. Consistent with the results of the previous large retrospective analyses, our study confirmed and externally validated a lack of association between the use of ABO-mismatched grafts and OS among patients who underwent related SCTs. In contrast, we found marginally significant impact of minor and bidirectional mismatch among those who received unrelated SCTs. This observation suggested the need for larger studies focusing on unrelated SCTs that include various ethnic backgrounds as the next step in assessing the clinical significance of ABO mismatching in SCTs.

In this study, the adverse impact of minor and bidirectional mismatch on OS after unrelated SCTs was observed in the following stratified categories: patients with acute leukemia, patients who received SCT after 1998, and patients who underwent transplants at Asian centers. These associations might

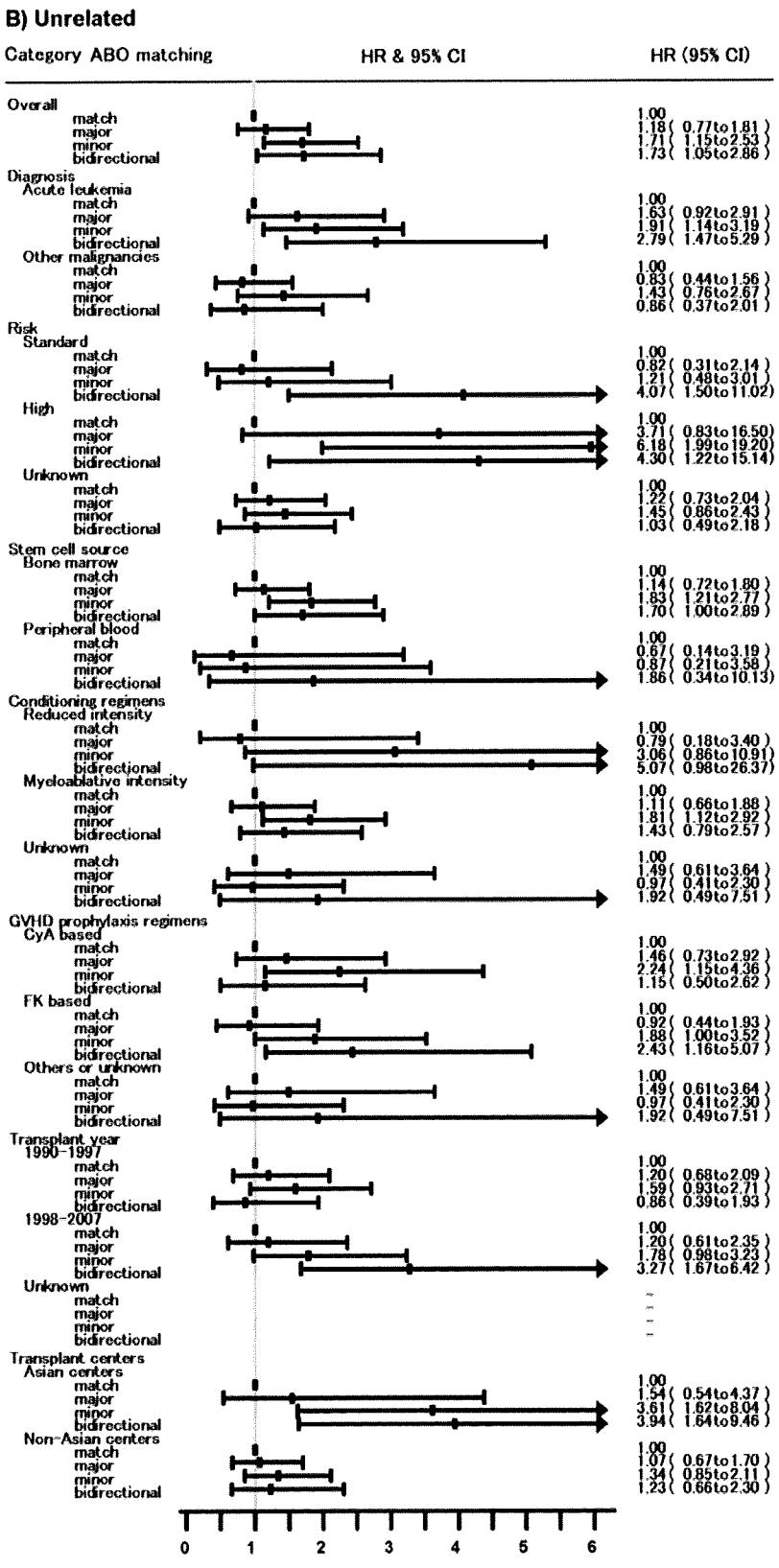

Fig. 3. Continued.

be biased by the relatively small size of unrelated transplant recipients in our analysis, because the previous study on the effect of ABO compatibility in unrelated SCTs among non-Asian populations reported that OS was not influenced by ABO mismatching.[21] However, more recently, a retrospective analysis of more than 5000 HLA-matched or mismatched unrelated SCTs facilitated by the Japan Marrow Donor Program revealed that the major ABO-mismatched group as well as minor mismatched group had inferior OS when compared to the ABO-matched group.[32] These varying results may partly be attributable to differences in the genetic backgrounds between Asian and non-Asian populations, such as cytokine gene polymorphisms and minor histocompatibility antigens:[33] it might be possible that the impact of minor and bidirectional mismatch is amplified by the increased immune dysregulation more likely to be seen in unrelated transplants compared with related transplants. Otherwise, ABO mismatching may exacerbate any underlying tendency toward complications seen in allogeneic transplantation, and these effects might be more prominent in unrelated SCTs. Recently, Michallet and coworkers[27] reported the results of a large retrospective study using the transplant data registered at the Société Française de Greffe de Moëlle et de Thérapie Cellulaire registry. The study analyzed 1108 patients who received related or unrelated SCTs after reduced-intensity conditioning for hematologic malignancies and it showed that minor ABO-mismatched grafts were associated with poorer OS. Although the background of patient characteristics in their study was different from that in this study, these results partly support our observation that minor and bidirectional mismatched grafts could have an adverse impact on OS.

However, the mechanism that underlies inferior survival after minor and bidirectional mismatched SCTs is presently unknown. In minor or bidirectional mismatched SCTs with marrow or peripheral blood grafts, passenger

TABLE 3. Impact of ABO mismatching on early TRM within 100 days and overall TRM

	Treatment-related death within 100 days (n = 1026)		Treatment-related death (n = 1026)	
Category	HRs (95% CI)*	p Value	HRs (95% CI)*	p Value
Overall				
Match	1.00		1.00	
Major	1.40 (0.84-2.32)	0.19	0.85 (0.57-1.28)	0.45
Minor	0.91 (0.52-1.59)	0.71	0.94 (0.65-1.34)	0.73
Bidirectional	2.08 (1.14-3.79)	0.017	1.45 (0.91-2.29)	0.11
Related SCT				
Match	1.00		1.00	
Major	1.10 (0.59-2.06)	0.75	0.81 (0.51-1.27)	0.36
Minor	0.81 (0.41-1.62)	0.56	0.85 (0.54-1.31)	0.45
Bidirectional	2.08 (1.04-4.15)	0.038	1.58 (0.95-2.64)	0.08
Unrelated SCT				
Match	1.00		1.00	
Major	2.10 (0.70-6.29)	0.19	0.84 (0.33-2.18)	0.72
Minor	1.17 (0.36-3.84)	0.79	1.15 (0.53-2.50)	0.72
Bidirectional	3.35 (0.95-11.80)	0.059	1.57 (0.63-3.92)	0.33

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

donor B lymphocytes are known to often produce anti-recipient hemagglutinin 1 or 2 weeks after SCT.[10-12,34] For certain periods of time, such hemagglutinin could be continuously absorbed on widely expressed A/B antigens in tissues and residual RBCs of the recipient. Therefore, in addition to complication of delayed hemolysis, production of immune complexes on the surfaces of recipient tissues shortly after SCT could be a target for alloreaction or could dysregulate immunity. In addition, different transfusion policies may affect survival in minor and bidirectional mismatched transplants, because Benjamin and Antin[35] suggested that the transfusion of plasma containing anti-A,B antibodies in group O PLTs and RBC may exacerbate the cytokine storm that follows allogeneic transplant. Assessing the number of components transfused and the presence and/or development of anti-A/B antibodies would be a worthwhile consideration in future studies.

Subgroup analyses regarding TRM and engraftment were performed with available data sets to evaluate other effects of ABO mismatching. Those analyses showed that the use of bidirectional mismatched grafts was associated with an increased risk of early TRM when compared with matched grafts ($p = 0.017$), while the overall TRM was similar. The higher TRM observed in the early period after bidirectional ABO-mismatched SCTs may be due to the combination of major and minor ABO mismatching with additive or synergistic enhancement of single adverse effects. Theoretically, major ABO mismatching leads to antidonor cell damage and release of cytokines soon after transplantation. That may enhance the subsequent activation of antihost donor-derived lymphocytes in the minor mismatch direction. Therefore, fatal transplant complications such as severe acute GVHD may occur more often among the bidirectional mismatched group.[18] This hypothesis was supported by our observation that the incidence of GVHD-related death within 100 days was significantly higher among recipients of bidirectional mismatched SCTs ($p < 0.001$). Furthermore, delayed engraftment of neutrophils and PLTs could potentially affect early transplant complications, such as infection and bleeding, although we could not clearly identify an increased risk of such complications among a subgroup of patients who received bidirectional mismatched grafts from an unrelated donor. To assess the effect of immunologic reactions between ABO-mismatched pairs, the genotype of genes regulating the secretor status of ABO substances and glycosyltransferases are worth exploring in future studies. First, it is well known that only "secretors," that is, individuals who possess the appropriate secretor genotype, can secrete the soluble H and ABO substances into the body fluids and plasma. In secretor patients, hemagglutinin may form immune complexes with secreted ABO substances in circulation. In contrast, in nonsecretor patients, it may react with the endothelial compartment as well as blood cells. These different immune reactions can modify treatment-related complications. Second, Eiz-Vesper and coworkers[36] have recently demonstrated that a genotype mismatch with regard to glycosyltransferases among phenotypically ABO-matched donor-recipient pairs can induce an alloreaction in vitro. Therefore, the genotypic difference may be a source of minor histocompatibility antigens and affect the risk of GVHD in addition to ABO mismatching.

Reticulocyte engraftment tended to be delayed for the major and bidirectional mismatched groups among recipients of unrelated SCTs ($p = 0.010$ and 0.012, respectively), consistent with previous reports.[2-6] The delay in reticulocyte engraftment may become more evident through the enhanced host-versus-graft reactions in some unrelated SCTs than in related SCTs. In addition, neutrophil and PLT recovery tended to be delayed among patients receiving bidirectional mismatched unrelated grafts ($p = 0.019$). Late recovery of neutrophils after ABO-mismatched transplantation was also observed in the major mismatched group of both related and unrelated SCTs,[18,24,37] although these findings were not confirmed in the present study. Rozman and colleagues[24] hypothesized that immune complexes formed after ABO-mismatched transplantation can cause a pseudo-delay in neutrophil engraftment because immune complexes can be constantly recognized by the Fc receptors on immune cells,

TABLE 4. Impact of ABO mismatching on reticulocyte, neutrophil, and PLT engraftment

	Reticulocytes (>1%) (n = 269)			Neutrophils (0.5×10^9/L) (n = 667)			PLTs ($>20 \times 10^9$/L) (n = 662)		
	Median (day)	HRs (95% CI)*	p Value	Median (day)	HRs (95% CI)*	p Value	Median (day)	HRs (95% CI)*	p Value
Overall									
Match	10	1.00		16	1.00		18	1.00	
Major	26	0.67 (0.47-0.96)	0.029	16	1.01 (0.83-1.23)	0.92	21	0.91 (0.75-1.11)	0.37
Minor	20	0.91 (0.64-1.30)	0.61	16	0.93 (0.73-1.17)	0.51	19	0.85 (0.69-1.06)	0.15
Bidirectional	21	0.84 (0.58-1.21)	0.35	17	0.76 (0.56-1.03)	0.079	20	0.66 (0.45-0.96)	0.031
Related SCT									
Match	18	1.00		16	1.00		17	1.00	
Major	23	0.89 (0.57-1.39)	0.61	17	1.05 (0.83-1.31)	0.70	21	0.92 (0.74-1.14)	0.44
Minor	19	0.81 (0.51-1.29)	0.37	16	0.90 (0.68-1.19)	0.47	19	0.90 (0.70-1.16)	0.43
Bidirectional	18	1.17 (0.75-1.84)	0.49	16.5	1.02 (0.70-1.47)	0.93	17.5	0.78 (0.48-1.29)	0.34
Unrelated SCT									
Match	22	1.00		16	1.00		21.5	1.00	
Major	30	0.42 (0.21-0.81)	0.010	16	0.85 (0.54-1.33)	0.47	22	0.98 (0.65-1.48)	0.92
Minor	20	0.85 (0.47-1.53)	0.58	15.5	0.93 (0.62-1.40)	0.74	24.5	0.61 (0.40-0.93)	0.023
Bidirectional	26	0.43 (0.22-0.83)	0.012	18	0.52 (0.30-0.90)	0.019	25	0.47 (0.24-0.91)	0.024

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

including neutrophils, which are subsequently removed from circulation. Finally, it should be mentioned that the presence of HLA antibodies, HLA allelic mismatching, or infused stem cell doses in unrelated donor SCTs could affect engraftment. It is desirable to include these factors in future studies of unrelated SCTs.

Limitations of this study should be noted. First, our data sets included heterogeneous diseases and various transplant methods, which made it difficult to elucidate the factors potentially associated with OS among minor and bidirectional mismatched transplantations. Second, the existence of missing data may have biased the results. In addition, data regarding the secondary endpoints were not available in some data sets. Therefore, these endpoints should be cautiously interpreted. Third, since we collected IPD from 6 of 11 candidate studies, there might be a potential selection bias. The findings of the meta-analysis should be interpreted in reference to the other large studies. Fourth, we performed the meta-analysis of nonrandomized cohort studies, which might limit our interpretation due to the potential selection bias. However, truly randomized control trials for SCT have rarely been conducted. Fifth, generally speaking, the effect of multiple testing should be taken into account when we interpret secondary endpoints. Finally, missing data on HLA matching between related donors and recipients might reduce the statistical power in the analysis of related SCTs. However, with regard to unrelated SCTs (n = 285), exclusion of patients receiving SCT from HLA-mismatched unrelated donors (n = 8) did not alter the main result (data not shown).

In conclusion, our IPD-based meta-analysis demonstrates no adverse association between any type of ABO mismatching and survival in allogeneic SCTs for hematologic malignancies, although the possible association of minor or bidirectional ABO mismatching with lower OS was observed among recipients of unrelated SCTs. Larger studies focusing on the effects of ABO matching in unrelated SCTs from various ethnic backgrounds with complete HLA allele information are warranted.

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