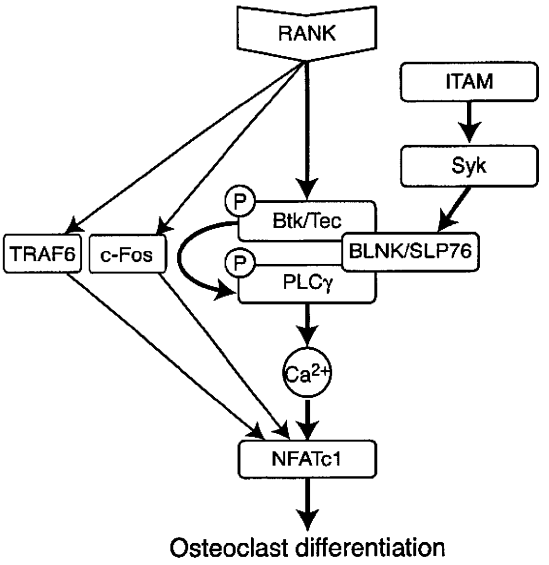

Figure 7. Integration of the RANK and ITAM Signals by Tec Kinases
RANKL binding to RANK results in activation of classical pathways involving TRAF6 and c-Fos. In addition, Tec kinases are phosphorylated by RANK. ITAM phosphorylation results in the recruitment of Syk, leading to activation of adaptor proteins such as BLNK and SLP-76, which function as scaffolds that recruit both Tec kinases and PLCγ to form the osteoclastogenic signaling complex. This complex is crucial for efficient activation of calcium signaling required for the induction and activation of NFATc1, the key transcription factor for osteoclast differentiation.

(Tsukada et al., 1993). Targeted disruption of *Btk* alone did not result in an obvious bone phenotype in mice, and B cell immunoglobulin production was also not severely affected in this strain (Kerner et al., 1995). Therefore, it is possible that the mouse and human utilization of Tec family kinases is not strictly the same. Because immunoglobulin production is completely abrogated in the combined deficiency of Btk and Tec (Ellmeier et al., 2000), $Tec^{-/-}Btk^{-/-}$ mice may serve as a better model of XLA. Currently there is no report on abnormalities of bone metabolism in the XLA patients, but it will be of great significance to analyze bone density and quality in patients with primary immunodeficiencies in the future. These studies, together with others, including recent data on hyper-IgM syndrome (Lopez-Granados et al., 2007), will surely shed light on unexpected aspects of the linkage between the immune and bone systems.

Upstream and Downstream of Tec Kinases in the Osteoclastogenic Signal Transduction

Although phosphorylation of Tec kinases is dependent on Src family kinases in immune cells (Schmidt et al., 2004), c-Src deficiency or inhibition of Src family kinases by PP2 has little effect on osteoclast differentiation (T.K. and H.T., unpublished data). Although it is possible that other Src family members may compensate, to date there has been no clear evidence demonstrating an essential role for Src kinases in osteoclast differentiation; therefore, the kinases that phosphorylate the Tec kinases in osteoclasts remain to be elucidated.

Based on the in vitro data (Figure 4B), the phosphorylation of PLCγ by RANKL is mostly dependent on Btk and Tec. However, osteopetrosis in $Tec^{-/-}Btk^{-/-}$ mice is less severe than that in $DAP12^{-/-}FcR\gamma^{-/-}$ mice, and pathological bone loss is not completely abrogated in the $Tec^{-/-}Btk^{-/-}$ mice or the Tec kinase inhibitor-treated mice. It is conceivable that the loss of Btk and Tec is partly compensated by other Tec kinases or that an alternative kinase(s) partially functions as a PLCγ kinase during osteoclastogenesis.

Despite the crucial role of BLNK and SLP-76 in osteoclast differentiation in vitro, bone mineral density was not markedly increased in $Blnk^{-/-}Lcp2^{-/-}$ mice (M.S., T.K., and H.T., unpublished data). One explanation for this discrepancy is that BLNK and SLP-76 do play a substantially important role, but another adaptor molecule, such as cytokine-dependent hematopoietic cell linker (Clnk), may compensate for the loss in vivo. Although further studies are necessary to elucidate the mechanism(s), it is likely that membrane-bound or soluble factors, which induce or activate Clnk, are compensatorily upregulated only in vivo. In addition, as $Blnk^{-/-}Lcp2^{-/-}$ mice develop acute leukemia at a very high frequency (more than 90%; D.K., unpublished data), the onset of acute leukemia may affect bone homeostasis by producing soluble factors that activate osteoclastogenesis or inhibit osteoblastic bone formation.

Tec Family Kinases as Therapeutic Targets for Bone Diseases

$Tec^{-/-}Btk^{-/-}$ mice are resistant to OVX-induced bone loss, but $DAP12^{-/-}FcR\gamma^{-/-}$ mice, which exhibit more severe osteopetrosis, do lose bone after OVX in certain bones (Wu et al., 2007). These results suggest that osteoclastogenesis under pathological conditions is dependent on a signaling mechanism distinct from that in physiological bone remodeling. Whatever the detailed mechanism, the results indicate that the Tec kinases offer some auspicious therapeutic targets in the treatment of metabolic and inflammatory bone diseases (see Figure 6).

Considering the severe immunodeficiency in XLA, careful attention would obviously have to be given to side effects on other cell types, including B cells, if Tec kinases were systemically inhibited in order to treat metabolic bone diseases. In the case of inflammatory bone diseases such as rheumatoid arthritis, certain immunosuppressants have already been successfully utilized in the clinic, so the inhibition of these kinases may prove to be a potentially effective strategy for preventing bone destruction associated with inflammation. Undoubtedly, the suppression of molecules shared by immune and bone cells will require a very careful evaluation in both systems prior to any clinical application, but these efforts will be rewarded by the provision of a molecular basis for novel drug design in the future.

EXPERIMENTAL PROCEDURES

Mice and Analysis of Bone Phenotype

We previously described the generation of $Tec^{-/-}Btk^{-/-}$ (Ellmeier et al., 2000) and $DAP12^{-/-}FcR\gamma^{-/-}$ (Koga et al., 2004) mice. $Blnk^{-/-}$ (Hayashi et al., 2003) and $Lcp2^{-/-}$ (Pivniouk et al., 1998) mice were described previously. Histomorphometric and microradiographic examinations were performed as described (Koga et al., 2004).

In Vitro Osteoclast Formation, Ca^{2+} Measurement, and GeneChip Analysis

Bone marrow cells were cultured with 10 ng/ml M-CSF (R & D Systems) for 2 days, and they were used as BMMs. BMMs were cultured with 50 ng/ml RANKL (Peprotech) and 10 ng/ml M-CSF for 3 days. RANKL and M-CSF were added at these concentrations unless otherwise indicated. In the coculture system, bone marrow cells were cultured with calvarial osteoblasts with 10^{-8} M 1,25-dihydroxyvitamin D_3 and 10^{-6} M prostaglandin E_2. TRAP-positive MNCs ($TRAP^+$ MNCs, more than three nuclei) were counted. Proliferation rate was determined 24 hr after RANKL stimulation using Cell Proliferation ELISA Kit (Roche). Apoptosis was assayed 24 hr after RANKL stimulation using In Situ Cell Death Detection Kit (Roche). In Figure 2D, LFM-A13 (Calbiochem) was added at the same time as RANKL. Concentration of intracellular calcium was measured and GeneChip analysis was performed as described (Takayanagi et al., 2002).

Retroviral Gene Transfer

Retroviral vectors, pMX-Tec-IRES-GFP, pMX-Btk-IRES-GFP, pMX-Btk (R28C)-IRES-GFP, and pMX-Btk (R525Q)-IRES-GFP, were constructed by inserting cDNA fragments of Tec, Btk, Btk (R28C), or Btk (R525Q) (Takata and Kurosaki, 1996) into pMX-IRES-EGFP. Retrovirus packaging was performed by transfecting Plat-E cells with the plasmids as described previously (Morita et al., 2000). After 6 hr inoculation, BMMs were stimulated with RANKL for 3 days.

Depletion of $CD19^+$ B Cells

Bone marrow cells contain $CD19^+$ cells at the ratio of about 30% in WT mice and about 15% in $Tec^{-/-}Btk^{-/-}$ mice. $CD19^+$ cells were depleted with a magnetic sorter and anti-CD19 microbeads (MACS; Miltenyi Biotec). The purity was confirmed by FACS, and the population of $CD19^+$ B cells was less than 5% in these preparations.

Immunoblot Analysis, Immunofluorescence Staining, and Flow Cytometry

After being stimulated with RANKL and M-CSF, BMMs were harvested and cell lysates were subjected to immunoblot or immunoprecipitation analyses with specific antibodies against Tec (Mano et al., 1995), Btk, NFATc1, PLCγ1, BLNK, DAP12, β-actin (Santa Cruz), phospho-PLCγ2, PLCγ2, phospho-ERK, ERK, phospho-p38, p38, phospho-JNK, JNK, phospho-Akt, Akt, phospho-IKKα/β, IKKα, IKKβ (Cell Signaling), phospho-PLCγ1 (Biosource International), Itk, 4G10 (Upstate), and phospho-Btk (BD Biosciences). For immunofluorescence staining, cells were fixed with 4% paraformaldehyde, permeabilized, and then treated with the indicated specific antibodies followed by staining with Alexa Fluor 488- or 546-labeled secondary antibody (Molecular Probes). For flow cytometry, bone marrow cells were incubated with the anti-CD11b antibody (BD Biosciences) or control rat IgG for 30 min followed by staining with PE-conjugated anti-rat IgG antibody.

Ovariectomy-Induced Bone Loss

Seven-week-old female mice were ovariectomized under anesthesia. Three or 8 weeks after surgery, all of the mice were sacrificed and subjected to histomorphometric and microradiographic examinations. Parameters for trabecular bone (thickness, separation, and number) were calculated on the basis of data obtained from microcomputed tomography analysis as described (Aoki et al., 2006).

LPS-Induced Bone Destruction

Seven-week-old female mice were administered with a local calvarial injection of LPS (Sigma) at 25 mg/kg body weight with a simultaneous injection of LFM-A13 (20 mg/kg body weight) or saline and were analyzed after 5 days as described (Takayanagi et al., 2000). For the detection of IKK phosphorylation, the calvarial tissues were homogenized 15 min after LPS injection, and lysates were subjected to immunoblot analysis. The serum levels of TNF-α and IL-6 (30 min after LPS injection) were measured by ELISA kits (R & D Systems).

RANKL-Induced Bone Loss

Seven-week-old C57BL/6 female mice were intraperitoneally injected with 20 μg of GST or GST-RANKL (Oriental Yeast Co., Ltd.) three times at intervals of 24 hr. LFM-A13 (20 mg/kg body weight) or saline was injected 1 hr prior to GST-RANKL treatment. One and a half hours after the final injection, all of the mice were sacrificed and subjected to histomorphometric and microradiographic examinations. The serum level of Ca^{2+} was measured by Calcium C (Wako).

Statistical Analysis

All data are expressed as the mean ± SEM (n = 5). Statistical analysis was performed by using Student's t test or ANOVA followed by Bonferroni test when applicable (*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; n.s., not significant). Results are representative examples of more than four independent experiments. In Figure 6, statistical analysis was performed between WT and $Tec^{-/-}Btk^{-/-}$ mice (n = 5 per group) on the fold increase of each parameter.

SUPPLEMENTAL DATA

Supplemental Data include six figures and can be found with this article online at http://www.cell.com/cgi/content/full/132/5/794/DC1/.

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Expansion of donor-derived hematopoietic stem cells with PIGA mutation associated with late graft failure after allogeneic stem cell transplantation

Kanako Mochizuki, Chiharu Sugimori, Zhirong Qi, Xuzhang Lu, Akiyoshi Takami, Ken Ishiyama, Yukio Kondo, Hirohito Yamazaki, Hirokazu Okumura and Shinji Nakao

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Brief report

Expansion of donor-derived hematopoietic stem cells with *PIGA* mutation associated with late graft failure after allogeneic stem cell transplantation

*Kanako Mochizuki,[1] *Chiharu Sugimori,[1] Zhirong Qi,[1] Xuzhang Lu,[1] Akiyoshi Takami,[1] Ken Ishiyama,[1] Yukio Kondo,[1] Hirohito Yamazaki,[1] Hirokazu Okumura,[1] and Shinji Nakao[1]

[1]Cellular Transplantation Biology, Division of Cancer Medicine, Kanazawa University Graduate School of Medical Science, Ishikawa, Japan

A small population of CD55$^-$CD59$^-$ blood cells was detected in a patient who developed donor-type late graft failure after allogeneic stem cell transplantation (SCT) for treatment of aplastic anemia (AA). Chimerism and *PIGA* gene analyses showed the paroxysmal nocturnal hemoglobinuria (PNH)–type granulocytes to be of a donor-derived stem cell with a thymine insertion in *PIGA* exon 2. A sensitive mutation-specific polymerase chain reaction (PCR)–based analysis detected the mutation exclusively in DNA derived from the donor bone marrow (BM) cells. The patient responded to immunosuppressive therapy and achieved transfusion independence. The small population of PNH-type cells was undetectable in any of the 50 SCT recipients showing stable engraftment. The de novo development of donor cell–derived AA with a small population of PNH-type cells in this patient supports the concept that glycosyl phosphatidylinositol–anchored protein–deficient stem cells have a survival advantage in the setting of immune-mediated BM injury. (Blood. 2008;112:2160-2162)

Introduction

Although small populations of CD55$^-$CD59$^-$ blood cells are often detectable in patients with aplastic anemia (AA), it remains unclear how such paroxysmal nocturnal hemoglobinuria (PNH)-type cells arise.[1] We recently encountered a patient with immune-mediated late graft failure (LGF) following allogeneic stem cell transplantation (SCT) for treatment of AA. Analyses of the patient's peripheral blood (PB) and bone marrow (BM) showed hematopoietic stem cells (HSCs) of donor origin with mutant *PIGA*, supporting the concept that glycosyl phosphatidylinositol–anchored protein (GPI-AP)–deficient stem cells have a survival advantage in the setting of immune mediated BM injury.

Methods

Patients

A 59-year-old man underwent allogeneic PBSCT from a human leukocyte antigen (HLA)–matched sibling donor after conditioning with fludarabin (120 mg/m^2), cyclophosphamide (1200 mg/m^2), and antithymocyte globulin (60 mg/kg) for treatment of very severe AA in April 2002 (Table 1) and achieved complete donor chimerism with normal blood cell counts. In January 2006, he developed pancytopenia and was diagnosed as having LGF without residual recipient cells. The patient underwent a second PBSCT from the original donor without preconditioning on February 8, 2006. Pancytopenia resolved completely by day 16 after PBSCT. However, at approximately day 60, the blood counts decreased gradually, and the patient became transfusion-dependent. On day 196 after the second PBSCT, the white blood cell (WBC) count was 5.3 × 10^9/L with 17% neutrophils, the hemoglobin concentration was 75 g/L, and the platelet count was 22 × 10^9/L. Treatment with horse antithymocyte globulin (ATG) and cyclosporine was started on day 205 after the second PBSCT. Transfusions were terminated after 88 days of the immunosuppressive therapy. Although the patient presently receives low-dose tacrolimus for treatment of chronic graft-versus-host disease, which developed 1 year after the second PBSCT, his pancytopenia has markedly improved as shown in Table 1. PB and BM of the patient were subjected to analyses of chimerism and flow cytometry to detect CD55$^-$CD59$^-$ cells and *PIGA* gene analysis.

As controls, the PB from 51 SCT recipients (48 with hematologic malignancies and 3 with AA) who achieved a complete recovery of donor-derived hematopoiesis were subjected to flow cytometric analysis for the screening of CD55$^-$CD59$^-$ cells. Of the 51 patients, 4 and 23, respectively, had acute graft-versus-host disease (GVHD) of grade II or higher and chronic GVHD at sampling.

BM aspirates were obtained from the patient's donor and 10 healthy individuals for *PIGA* gene analysis. Informed consent was obtained from all patients and healthy individuals in accordance with the Declaration of Helsinki for blood examination, and the experimental protocol for *PIGA* gene analysis was approved by our participating institutional ethics committee (No.157).

Detection of PNH-type cells

To detect GPI-AP deficient (GPI-AP$^-$), PNH-type cells, we performed high-sensitivity 2-color flow cytometry of granulocytes and red blood cells (RBCs), as described previously.[1] The presence of 0.003% or more CD55$^-$CD59$^-$CD11b$^+$ granulocytes and 0.005% or more CD55$^-$CD59$^-$glycophorin-A$^+$ RBCs was defined as an abnormal increase based on the results in 183 healthy individuals.[2]

Cell sorting and chimerism analysis

CD3$^+$cells were isolated from the PB mononuclear cells of the patient using magnetic-activated cell sorting (MACS) CD3 Microbeads (Miltenyi Biotec, Auburn, CA). The CD55$^-$CD59$^-$CD11b$^+$ granulocytes were separated from the CD55$^+$CD59$^+$CD11b$^+$ granulocytes with a cell sorter (JSAN; Bay Bioscience, Yokohama, Japan). More than 95% of the sorted cells were

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*K.M. and C.S. contributed equally to this work.

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Table 1. Hematologic parameters of donor and recipient

	Donor		Recipient			
			Before 1st SCT	Before 2nd SCT	At ATG therapy	After 20 mo of ATG therapy
Date	Apr 2002	May 2008	Apr 2002	Jan 2006	Aug 2006	Apr 2008
WBC count, × 10^9/L	7.0	5.1	1.2	1.7	5.3	4.0
Neutrophil proportions, %	77	65	0	0	17	62
RBC count, × 10^{12}/L	4.21	4.43	2.20	2.75	2.07	3.04
Reticulocytes, × 10^9/L	not tested	35	2	3	26	61
Hemoglobin, g/L	146	150	72	89	75	120
Platelet count, × 10^9/L	261	230	19	52	22	54

$CD55^-CD59^-CD11b^+$. The *D1S80* locus was amplified from DNA of different cell populations with an AmpliFLP D1S80 PCR Amplification Kit (Perkin-Elmer Cetus, Norwalk, CT).

PIGA gene analysis

The coding regions of *PIGA* were amplified by seminested PCR or nested PCR from DNA extracted from the sorted PNH-type cells using 12 primer sets[3,4] (Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article), and 6 ligation reactions were used to transform competent *Escherichia coli* JM109 cells (Nippon Gene, Tokyo, Japan). Five clones were selected randomly from each group of transfectants and subjected to sequencing with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Amplification refractory mutation system PCR

On the basis of a mutant sequence detected in *PIGA* of the patient, a nested amplification refractory mutation system (ARMS) forward primer with a 3′-terminal nucleotide sequence complementary to the mutant sequence was prepared[5] (Table S1). To enhance the specificity, a mismatch at the penultimate nucleotide position of the mutation site was incorporated in the ARMS forward primer (P1).[6,7] P1 and a reverse primer (P3) were used to amplify a 127 bp fragment containing the mutant sequence from the exon 2 amplified product. PCR was conducted under the following conditions; denaturation for 30 seconds at 94°C, annealing for 60 seconds at 64°C and extension for 90 seconds at 72°C for 20 cycles. Another forward primer (P2), complementary to the wild-type *PIGA* sequence upstream of the mutation site, was used in combination with P3 to amplify an internal control according to the same condition of ARMS-PCR.

Results and discussion

PNH-type cells were not detected in the donor or the patient at the time of development of the first LGF, whereas 0.147% PNH-type granulocytes and 0.019% PNH-type RBCs were detected in the PB

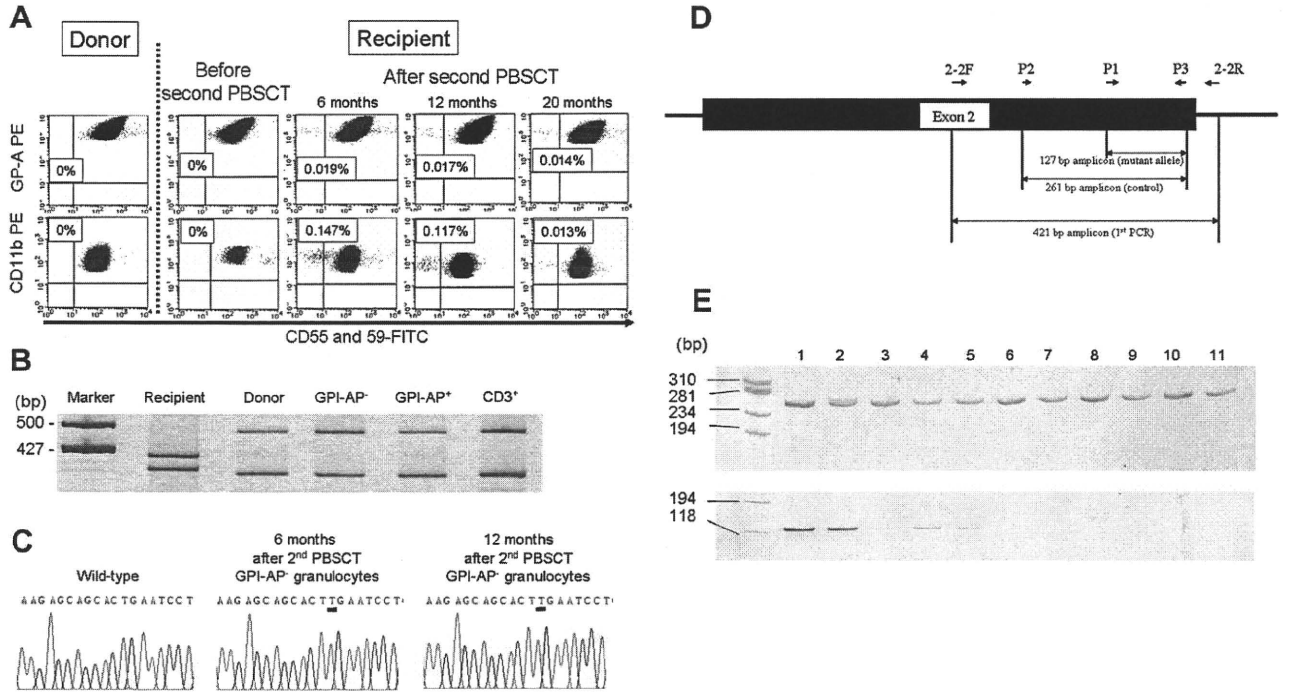

Figure 1. Analysis of PNH-type cells after the second PBSCT. (A) High-sensitivity flow cytometry detected small populations of $CD55^-CD59^-$cells in both granulocytes and red blood cells at the development of the second LGF as well as in those obtained 6 and 12 months later, but did not detect PNH-type cells in the donor or in the recipient before the second PBSCT. The numbers denote the proportion of PNH-type cells in $CD11b^+$ granulocytes or glycophorin A^+ RBCs. (B) *D1S80* allelic patterns of sorted $GPI\text{-}AP^-$ granulocytes, $GPI\text{-}AP^+$ granulocytes, and $CD3^+$ lymphocytes. The polymerase chain reaction (PCR) products were subjected to 8% polyacrylamide gel electrophoresis and visualized by silver staining. (C) Nucleotide sequences of *PIGA* exon 2 in DNA from PNH-type granulocytes obtained 6 and 12 months after the second PBSCT. (D) A schematic illustration for ARMS-PCR is shown. Primer positions for the first, second are shown by short arrows. A black box and adjacent lines represent exon 2 and introns, respectively. (E) Amplified products of control PCR (the upper gel) and ARMS-PCR (the lower gel) were electrophoresed in 12.5% polyacrylamide gel and visualized by the silver staining. A pMD20-T vector containing the mutated exon 2 fragment was used as a positive control for ARMS-PCR. The template DNA derives from a plasmid containing the mutated exon 2 in lane 1, donor BM in lane 2, donor PB in lane 3, recipient BM in lane 4, recipient PB in lane 5, and BM from healthy individuals in lanes 6 to 11. PCR with a 5′ primer specific to the nucleotide sequence upstream of the mutated sequence amplified a 261 bp fragment from DNA of the donor and all healthy individuals.

obtained at the time of development of the second LGF (Figure 1A). Similar percentages of PNH-type blood cells were detectable in the PB of the patient 6 and 14 months later. When PB from 51 SCT recipients was examined, none of the patients were found to have detectable PNH-type cells (data not shown). PNH-type blood cells were also undetectable in a donor PB sample obtained 21 months later.

The *D1S80* locus allelic pattern of the PNH-type granulocytes in the patient was compatible to that of the donor (Figure 1B). The emergence of donor-derived PNH-type cells and hematologic improvement after immunosuppressive therapy suggest that LGF arises as a result of de novo development of AA which affects the donor-derived hematopoietic stem cells (HSCs).

PIGA gene analysis of the DNA prepared from the sorted PNH-type cells of the patient obtained at the development of LGF and 6 months later showed an insertion of thymine at position 593 (codon 198) in 3 of 5 clones and 5 of 5 clones examined, respectively (Figure 1C). Mutations in other exons were not identified. The presence of a single *PIGA* mutation in PNH-type granulocytes and its persistence over 6 months suggest that these PNH-type cells are derived from a mutant HSC rather than from a committed granulocyte progenitor cell. Moreover, an ARMS-PCR with a 5′ primer specific to the mutated sequence amplified a 127 bp fragment from DNA of the donor BM as well as of the recipient BM and PB while it failed to amplify the same fragment in donor PB and in BM of all 10 healthy individuals (Figure 1D).

These experiments demonstrate that *PIGA*-mutant HSCs were present in the BM of the donor in a dormant state and were transplanted into the recipient and provide, for the first time, in vivo evidence that *PIGA* mutant, GPI-AP–deficient HSCs have a survival advantage in the setting of immune mediated BM injury. Similarly, relative resistance to immune injury likely accounts for the high incidence of PNH observed in association with acquired AA.

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Authorship

Contribution: K.M. and C.S. participated in designing and performing the research. Z.Q. and X.L. performed experiments. K.M., C.S., and S.N. wrote the paper. C.S., A.T., K.I., Y.K., H.Y., and H.O. provided patient care. All authors have approved the final version of the manuscript.

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Correspondence: Shinji Nakao, Cellular Transplantation Biology, Division of Cancer Medicine, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan; e-mail: snakao@med3.m.kanazawa-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,

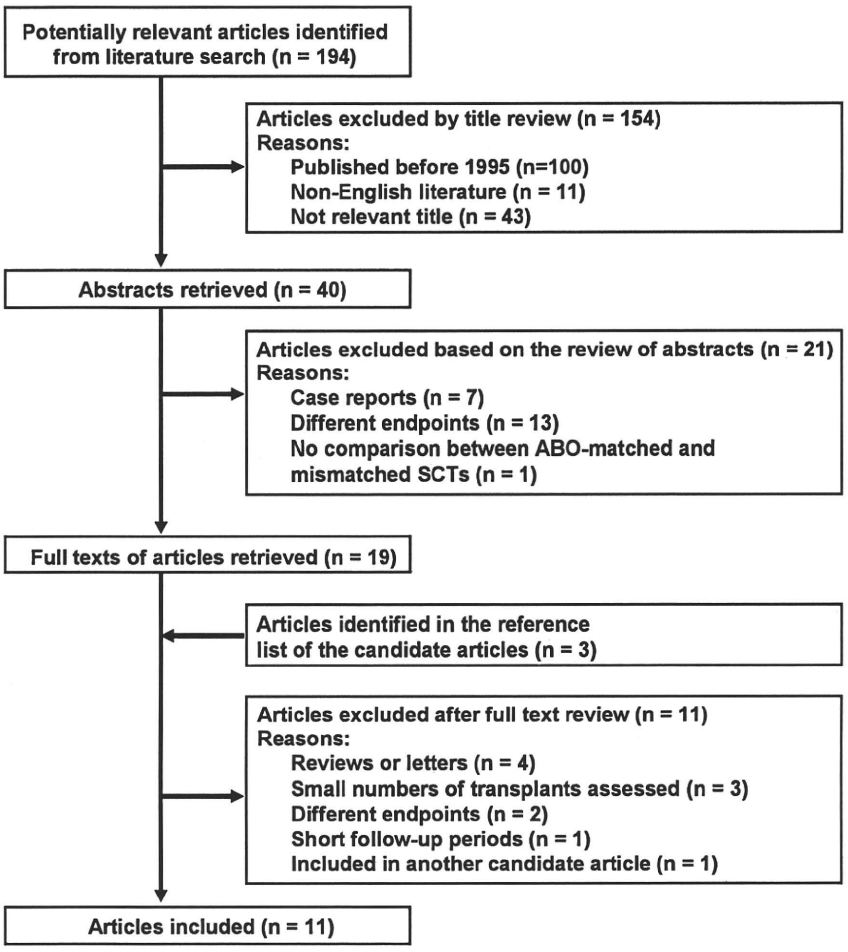

Fig. 1. A flow chart illustrating the process of article selection.

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

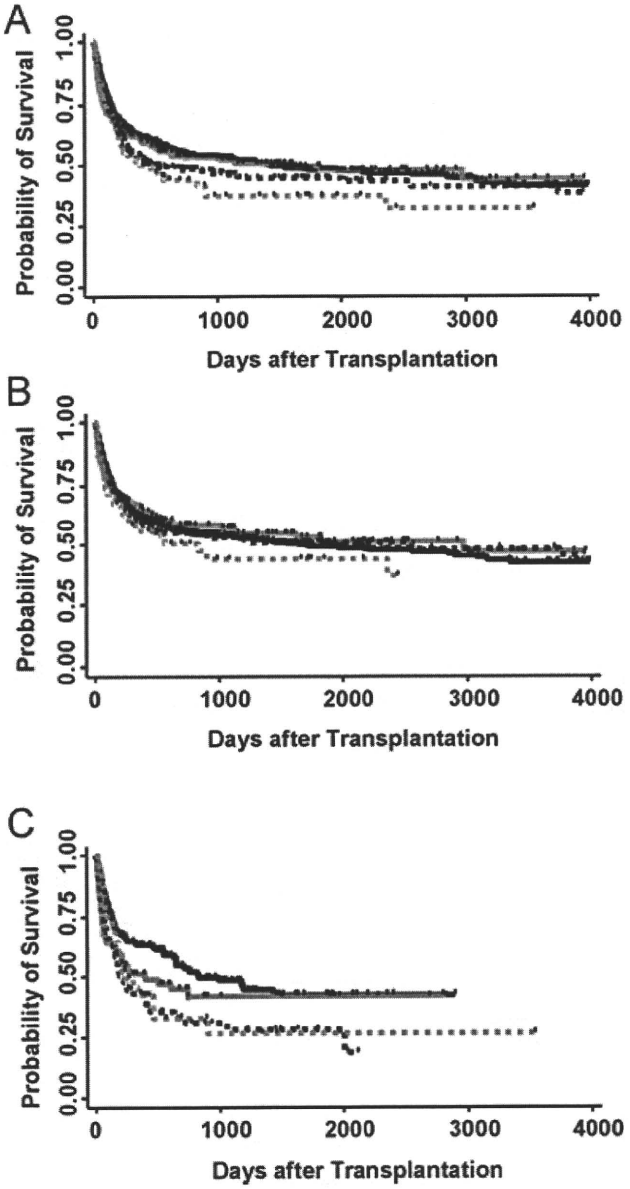

Fig. 2. Kaplan-Meier survival estimates of OS in all patients (A), those who received a related graft (B), and those who received an unrelated graft (C). (—) ABO-matched transplantation; (—) major mismatched; (· · ·) minor mismatched; (-···) bidirectional mismatched.

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).

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.

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.

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,

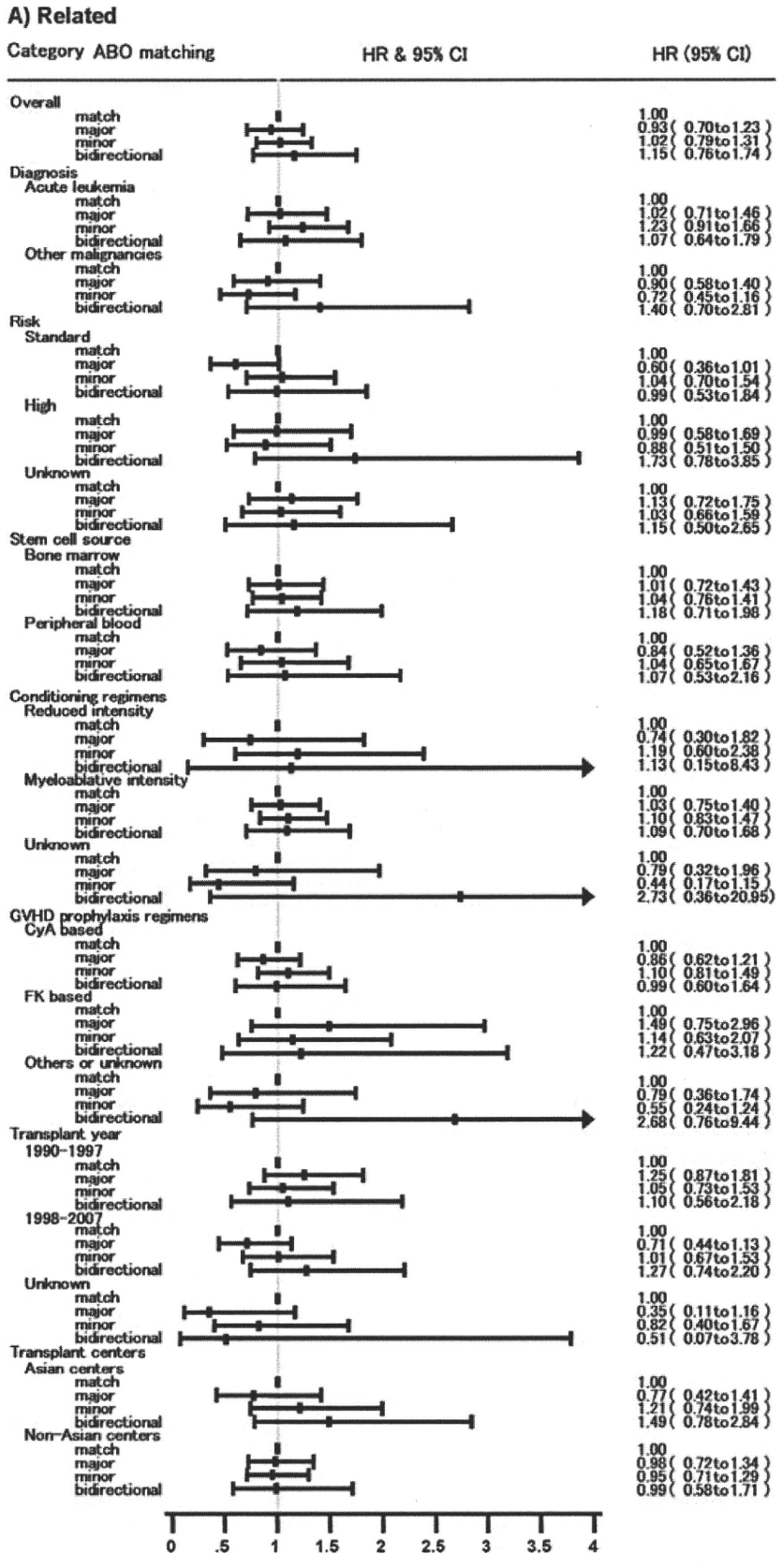

Fig. 3. Impact of ABO mismatching on OS in each stratified category among related (n = 923) (A) and unrelated stem cell transplantation (n = 285) (B). HRs were adjusted for age and sex. Square boxes on lines indicate HRs, and horizontal lines represent 95% CI.

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

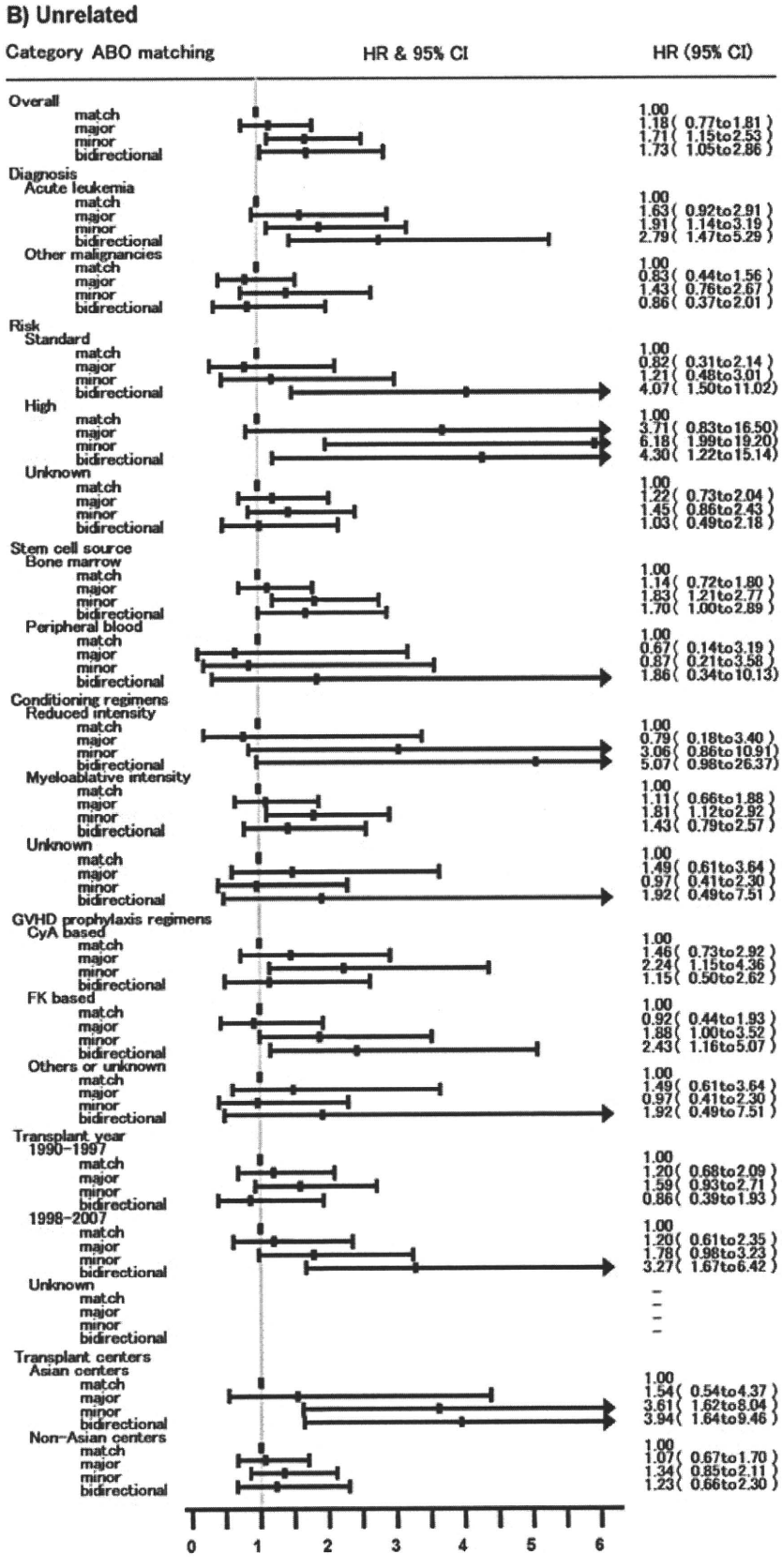

Fig. 3. Continued.

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

Category	Treatment-related death within 100 days (n = 1026) HRs (95% CI)*	p Value	Treatment-related death (n = 1026) 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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Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study

Masakatsu Hishizawa, Junya Kanda, Atae Utsunomiya, Shuichi Taniguchi, Tetsuya Eto, Yukiyoshi Moriuchi, Ryuji Tanosaki, Fumio Kawano, Yasushi Miyazaki, Masato Masuda, Koji Nagafuji, Masamichi Hara, Minoko Takanashi, Shunro Kai, Yoshiko Atsuta, Ritsuro Suzuki, Takakazu Kawase, Keitaro Matsuo, Tokiko Nagamura-Inoue, Shunichi Kato, Hisashi Sakamaki, Yasuo Morishima, Jun Okamura, Tatsuo Ichinohe and Takashi Uchiyama

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